In the Name of God

Recent Topics in Horticulture

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Trends in Fruit Breeding

1-Environmental Issues

آلودگی زیست محیطی	گرم شدن جهانی زمین	کشاورزی پایدار

2-Health Consciousness

- میوه سالم تر
- میوه با ارزش غذایی بالاتر
- توسعه کشاورزی پایدار و ارگانیک

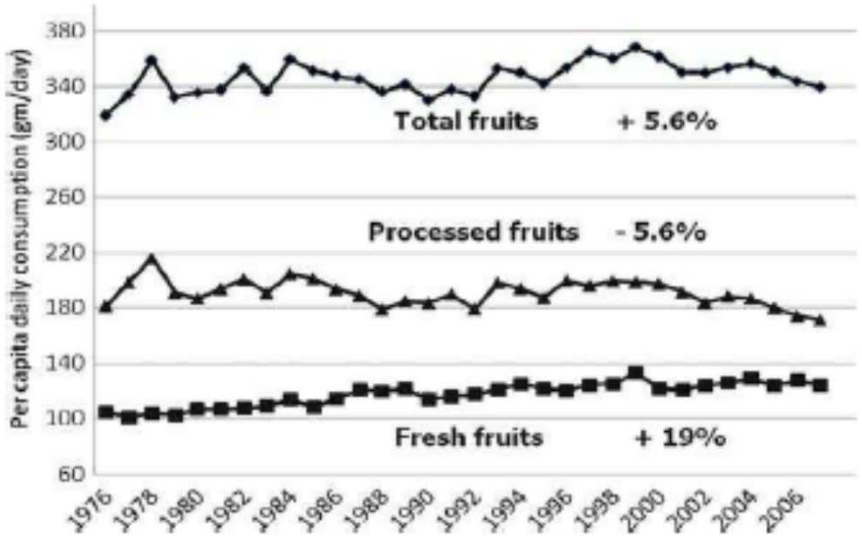


Fig. 1.1 Per capita fruit consumption in the USA (data from Pollack and Perez 2008)

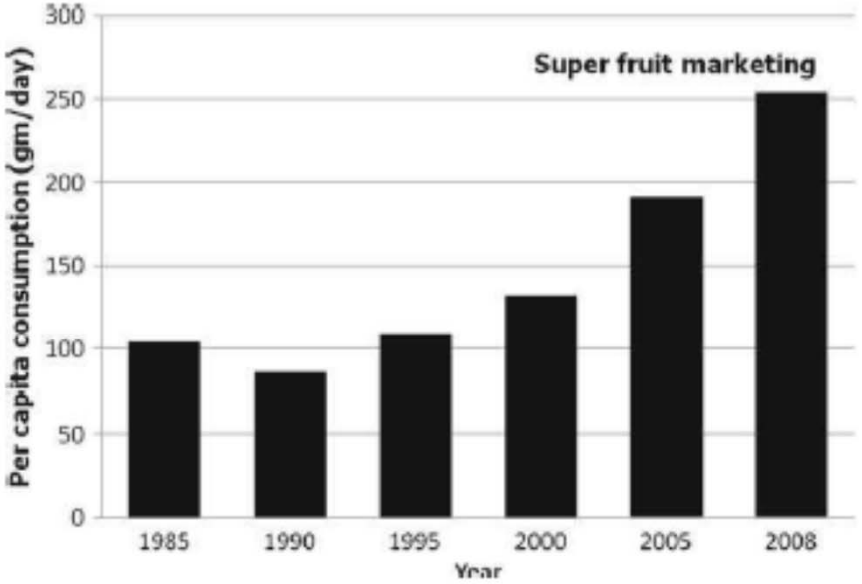


Fig. 1.2 Per capita blueberry consumption in the USA (data from Pollack and Perez 2008)

Health Benefits of Fruits

	Wild type fruits	Health enhanced cultivars

3-Consumer Expectations and Habits

- محصول ارگانیک (green)
- سهولت در مصرف، عطر و طعم خوب، کیفیت ثابت
- انبارمانی طولانی، تولید در سراسر سال

4-Producer Expectations: Simplified Management

- رقم های با بیشترین کمیت و کیفیت
- کاهش هزینه کارگری
- کاهش هزینه مواد شیمیایی

Fruit Breeding Goals

Simplifying Orchard Practices		
تغییر اندازه درخت یا عادت رشد	پایه های پاکوتاه پیوندک های spur type	افزایش سفتی میوه یکنواختی رسیدن سهولت در جدا شدن مقاومت به ضربه


Fruiting Stability
self fertility
مقاومت به آفات و بیماری ها
سازگاری به نواحی تولید جدید
مقاومت به تنش های محیطی

Table 1.4 Disease and pest problems of major tree fruit crops

Crop	Disease	Pathogen/pest	Comments
Pome fruit	Apple scab	*Venturia*	Genes/markers identified, many resistant apple cv.
	Powdery mildew	*Podosphaera*	Genes/markers identified, resistant apple cv.
	Fire blight	*Erwinia*	Active work, resistant apple/pear cv. and rootstock
	Black spot	*Stemphylium*	Little work, widespread on pear
	Psylla	*Cacopsylla*	Transmit pear decline
Stone fruit	Brown rot	*Monolinia* spp.	Little progress, some less susceptible cv.
	Bacterial leaf spot	*Xanthomonas*	Good progress, polygenic, resistant cv.
	Plum pox	*Potyvirus*	Genes/markers identified, active breeding, transgenic resistant plum
	Peach scab	*Cladosporium*	Little work, widespread problem
	Root knot nematodes	*Meloidogyne*	Genes/markers identified, resistant rootstocks
Citrus	Citrus greening	*Candidatus Liberibacter*	No resistance known
	Citrus canker	*Xanthomonas*	Tangerines moderately resistant, polygenic resistance
	Citrus tristeza virus	*Closterovirus*	Genes/markers identified, resistant rootstocks, active breeding
	Phytophthora	*Phytophthora*	Resistant rootstocks
	Nematodes	*Tylenchulus*	Genes/markers identified
Grapes	Powdery mildew	*Erysiphe*	Gene identified, active breeding
	Pierce's disease	*Xylella*	Gene identified, active breeding
	Nematodes	*Meloidogyne*	Dominant gene, resistant rootstocks
	Phylloxera	*Daktulosphaira*	Resistant rootstocks

Source: Brown (2003); Lespinasse (2009); Lespinasse et al. (2008); Fischer et al. (2003); Byrne (2005); Gmitter et al. (2007); Riaz et al. (2007); Ramming et al. (2009)

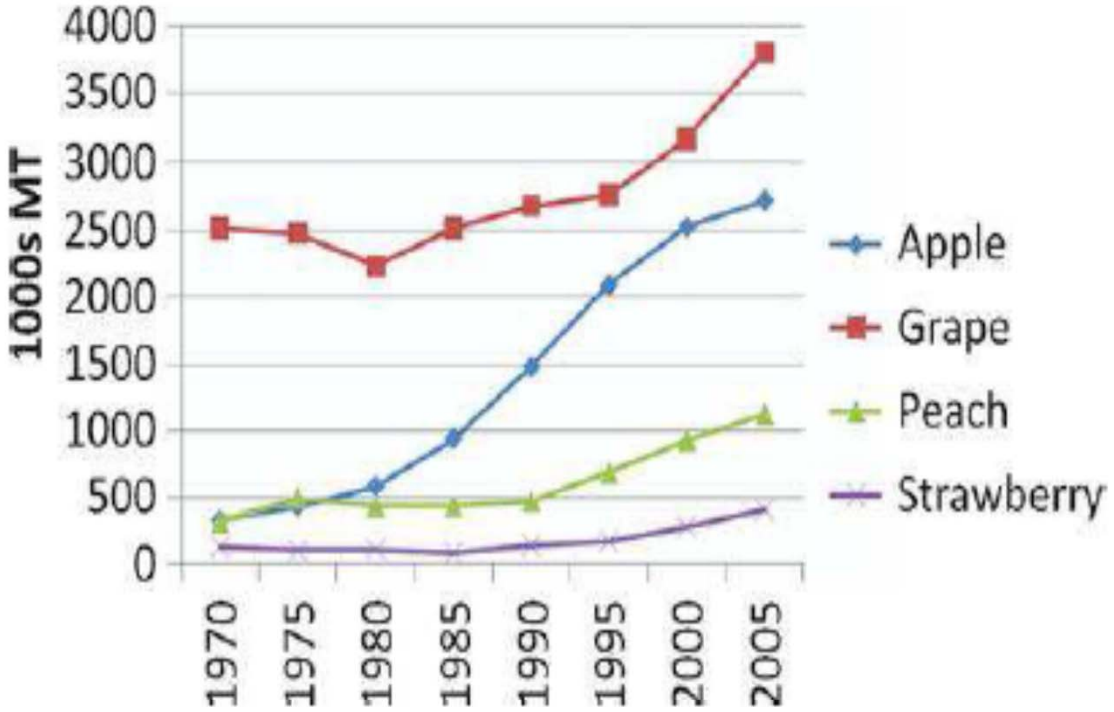
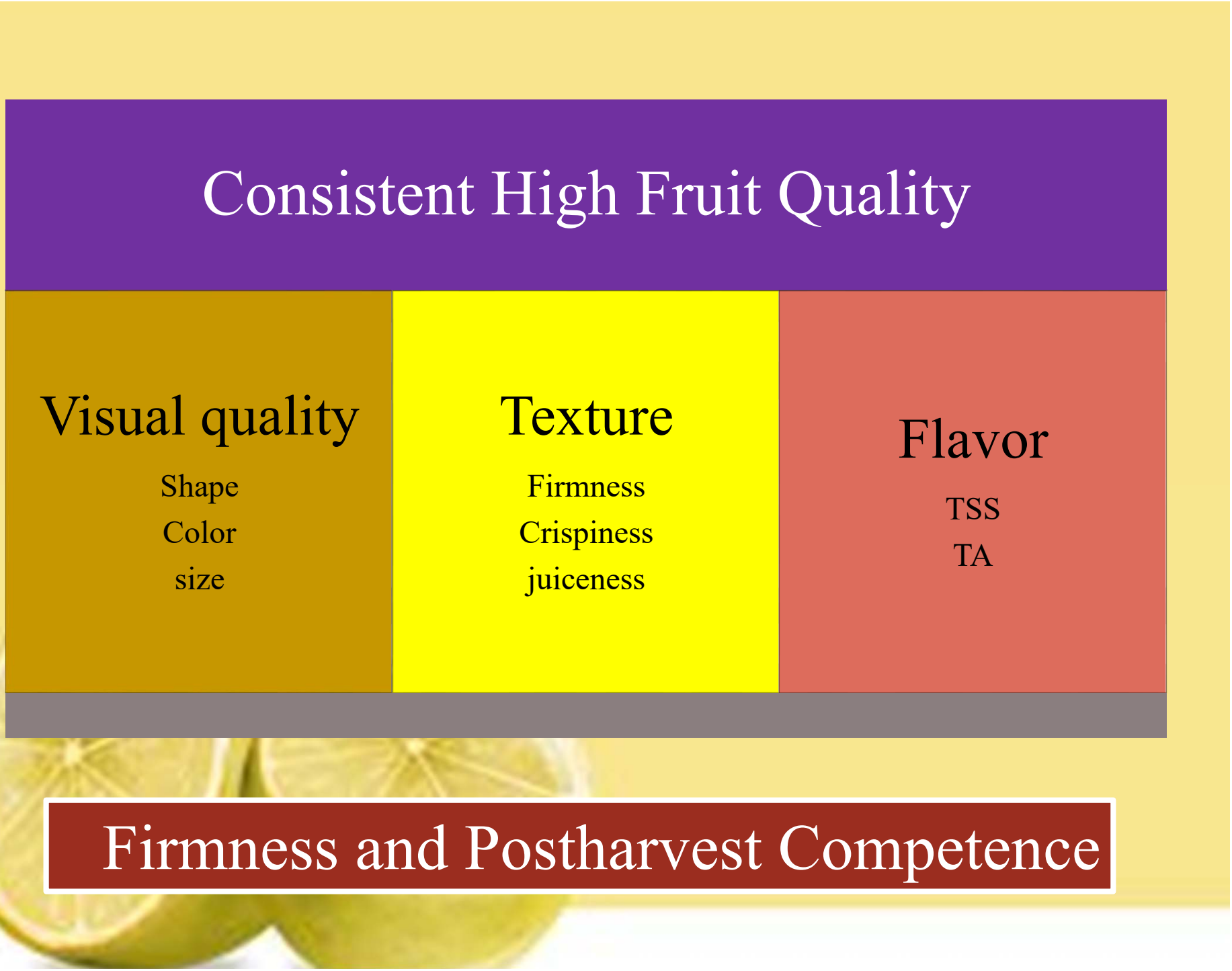


Fig. 1.3 Fruit production in medium- and low-chill zones of the Americas and Northern Africa

Diversification of fruit Types

عطر و طعم جدید	ظاهر جذاب	ویژگی کیفی متمایز


Consistent High Fruit Quality
Visual quality
Shape
Color
size
Texture
Firmness
Crispiness
juiceness
Flavor
TSS
TA
Firmness and Postharvest Competence

MICROGRAFTING

or

SHOOT-TIP GRAFTING (STG)

2012

Rank	Area	Production (Int $1000)	Flag	Production (MT)
1	[redacted]	3481071	*	18012560
2	[redacted]	1578237	*	8166480
3	[redacted]	1256177	*	6500000
4	[redacted]	966290	*	5000000
5	[redacted]	708636	*	3666790
6	[redacted]	566980	*	2933800
7	[redacted]	538493	*	2786397
8	[redacted]	342163	*	1770503
9	[redacted]	321194	*	1662000
10	[redacted]	311691	*	1612828
11	[redacted]	311490	*	1611784
12	[redacted]	275392	*	1425000
13	[redacted]	248336	*	1285000
14	[redacted]	185863	*	961738
15	[redacted]	173932	*	900000
16	[redacted]	155092	*	802517
17	[redacted]	152983	*	791600
18	[redacted]	120786	*	625000
19	[redacted]	104785	*	542207
20	[redacted]	100657	*	520845

2011

Rank	Area	Production (Int $1000)	Flag	Production (MT)
1	Brazil	3575879	*	18503139
2	United States of America	1445168	*	7477924
3	India	1153054	*	5966400
4	China, mainland	1047071	*	5418000
5	Mexico	783010	*	4051631
6	Spain	601960	*	3114800
7	Egypt	464015	*	2401015
8	Italy	462594	*	2393663
9	Indonesia	392101	*	2028904
10	Turkey	330567	*	1710500
11	Pakistan	290853	*	1505000
12	Iran (Islamic Republic of)	290431	*	1502819
13	South Africa	273379	*	1414585
14	Greece	174183	*	901300
15	Morocco	164114	*	849197
16	Argentina	161077	*	833486
17	Viet Nam	140962	*	729400
18	Syrian Arab Republic	129270	*	668900
19	Algeria	112572	*	582496
20	Ghana	112089	*	580000

2005

Rank	Area	Production (Int $1000)	Flag	Production (MT)
1	Brazil	3450320	*	17853443
2	United States of America	1622066	*	8393270
3	Mexico	794814	*	4112711
4	India	640457	*	3314000
5	China, mainland	492244	*	2547084
6	Spain	459225	*	2376230
7	Italy	437034	*	2261404
8	Iran (Islamic Republic of)	435450	*	2253209
9	Indonesia	427876	*	2214019
10	Egypt	375001	*	1940420
11	Pakistan	332597	*	1721000
12	Turkey	279257	*	1445000
13	South Africa	240887	*	1246454
14	Greece	180907	*	936094
15	Argentina	171201	*	885871
16	Morocco	161370	*	835000
17	Viet Nam	116206	*	601300
18	Ghana	96629	*	500000
19	Australia	96264	*	498112
20	Syrian Arab Republic	87468	*	452600

2000

Rank	Area	Production (Int $1000)	Flag	Production (MT)
1	Brazil	4122243	*	21330258
2	United States of America	2278643	*	11790680
3	Mexico	736831	*	3812683
4	India	516907	*	2674700
5	Spain	505605	*	2616220
6	Italy	362587	*	1876182
7	Iran (Islamic Republic of)	356283	*	1843564
8	Egypt	311245	*	1610520
9	Pakistan	256646	*	1328000
10	South Africa	226074	*	1169806
11	Turkey	206786	*	1070000
12	China, mainland	203693	*	1054000
13	Greece	182776	*	945765
14	Morocco	168134	*	870000
15	Argentina	152112	*	787096
16	Indonesia	124468	*	644052
17	Australia	98556	*	509973
18	Venezuela (Bolivarian Republic of)	96004	*	496768
19	Cuba	90925	*	470487
20	Viet Nam	82463	*	426700

1998

Rank	Area	Production (Int $1000)	Flag	Production (MT)
1	Brazil	4029526	*	20850504
2	United States of America	2396592	*	12401000
3	Mexico	643771	*	3331152
4	Spain	474523	*	2455390
5	India	454967	*	2354200
6	Iran (Islamic Republic of)	338044	*	1749185
7	Egypt	278610	*	1441652
8	Pakistan	251815	*	1303000
9	Italy	249994	*	1293580
10	Morocco	213318	*	1103800
11	China, mainland	207559	*	1074000
12	Argentina	190133	*	983833
13	Turkey	187460	*	970000
14	South Africa	186221	*	963589
15	Greece	157225	*	813553
16	Australia	96587	*	499784
17	Indonesia	94877	*	490937
18	Venezuela (Bolivarian Republic of)	91802	*	475023
19	Syrian Arab Republic	84832	*	438960
20	Viet Nam	77593	*	401500

سطح زیرکشت، میزان‌تولید و عملکرد مرکبات کشور به تفکیک استان در سال ۱۳۸۷

جدول شماره ۲-۳ ((واحد: هکتار))

نام استان	سطح کشت باغات (با احتساب درختان مخلوط و پراکنده)						
	غیربارور			بارور			جمع
	آبی	دیم	جمع	آبی	دیم	جمع	
ایلام	۳۲٤,۹	۰	۳۲٤,۹	٦٦,۹	۰	٦٦,۹	۳۹۱,۸
بوشهر	۸۳۱,۳	۰	۸۳۱,۳	۲۳۷۲,۸	۰	۲۳۷۲,۸	۳۲۰٤,۱
خوزستان	۱٦۸٤,۲	۰	۱٦۸٤,۲	٤۱۱۷	۰	٤۱۱۷	۵۸۰۱,۲
سیستان‌وبلوچستان	۱۰۲۷,۸	۰	۱۰۲۷,۸	۲۰۳۵,٦	۰	۲۰۳۵,٦	۳۰٦۳,٤
فارس	۹٦۷۱,۳	۰	۹٦۷۱,۳	۵۷۱۸۰,٦	۰	۵۷۱۸۰,٦	٦٦۸۵۱,۹
کرمان	۱۳۸۲	۰	۱۳۸۲	۱۳۱۹۹,۳	۰	۱۳۱۹۹,۳	۱٤۵۸۱,۳
کرمانشاه	۳	۰	۳	۳۱۵,۷	۰	۳۱۵,۷	۳۱۸,۷
کهگیلویه‌وبویراحمد	۱٦۲۷,٦	۰	۱٦۲۷,٦	۲۲٤۰,۲	۰	۲۲٤۰,۲	۳۸٦۷,۸
گلستان	۱٤۹۸,۸	۸	۱۵۰٦,۸	۳۷۰۵,۲	۰	۳۷۰۵,۲	۵۲۱۲
گیلان	۲۷,۳	٤۷۱٦	٤۷٤۳,۳	٤۱,۸	۱٤٦۰۲,۷	۱٤٦٤٤,۵	۱۹۳۸۷,۸
لرستان	٦٦,۵	۰	٦٦,۵	۳۵۷	۰	۳۵۷	٤۲۳,۵
مازندران	۱۳۰۱۷,٤	٦۸۸۷,٦	۱۹۹۰۵	٦۲۵۵۱,٦	۲۰۷۵٤,٦	۸۳۳۰٦,۲	۱۰۳۲۱۱,۲
هرمزگان	۳٤۹٤,۳	۰	۳٤۹٤,۳	۲۹۸۸۰,۳	۰	۲۹۸۸۰,۳	۳۳۳۷٤,٦
یزد	۳۷,۱	۰	۳۷,۱	۷۲,۸	۰	۷۲,۸	۱۰۹,۹
منطقه جیرفت و کهنوج	۳۷۰۷,۷	۰	۳۷۰۷,۷	۲۷۰٦۸,٦	۰	۲۷۰٦۸,٦	۳۰۷۷٦,۳
کل کشور	۳۸٤۰۱,۳	۱۱٦۱۱,٦	۵۰۰۱۲,۹	۲۰۵۲۰۵,۵	۳۵۳۵۷,٤	۲٤۰۵٦۲,۹	۲۹۰۵۷۵,۸

- میزان تولید:

تولید مرکبات کشور حدود ۴ میلیون تن برآورد شده و ۸۶,۳ درصد آن از اراضی آبی حاصل شده است.

در بین استان‌ها، بیشترین تولید مرکبات با ۴۵,۱ درصد از کل تولید این محصول در استان مازندران بوده است. استان‌های فارس، منطقه جیرفت و کهنوج، هرمزگان، گیلان و کرمان به ترتیب با ۲۷,۵، ۹,۷، ۹,۴، ۲,۵ و ۲,۱ درصد سهم در تولید مرکبات کشور در رتبه‌های بعدی قرار دارند. شش استان مزبور در مجموع ۹۶,۴ درصد مرکبات کشور را تولید کرده‌اند.

- عملکرد در هکتار:

راندمان تولید مرکبات آبی درکشور ۱۶۹۳۱,۶ کیلوگرم در هکتار است. بیشترین و کمترین عملکرد آبی به ترتیب با ۲۱۸۳۲,۲ و ۴۸۳,۷ کیلوگرم به استان‌های مازندران و لرستان تعلق دارد.

متوسط تولید در هکتار مرکبات دیم کشور ۱۵۵۶۱,۳ کیلوگرم می‌باشد. استان مازندران با تولید ۲۱۶۶۲,۲ کیلوگرم در هکتار بالاترین و استان گیلان با تولید ۶۸۹۰,۲ کیلوگرم در هکتار کمترین عملکرد دیم را داشته‌اند.

فهرست مطالب

بیماریهای مهم مرکبات

گردآوری و تالیف:
یعقوب محمدعلیان
حسین طاهری
فرید بیگی
رضا مقصودی

تهیه شده در واحد رسانه های ترویجی
زمستان ۱۳۸۴

بیماری تریستزای مرکبات

تریستزا به عنوان یکی از مخربترین بیماریهای ویروسی مرکبات، در بسیاری از کشورهای مرکبات خیز جهان باعث خسارت فراوان به این محصول گردیده است.

اولین اپیدمی بزرگ تریستزا در آرژانتین و برزیل اتفاق افتاد. این بیماری همراه با نهال های پیوندی نارنگی انشوی زودرس از ژاپن وارد ایران (باغ مهدشت ساری) گردید و پس از گذشت ۳۰ سال انتشار آن به وسیله شته سبز جالیز در شرق مازندران گزارش شده است.

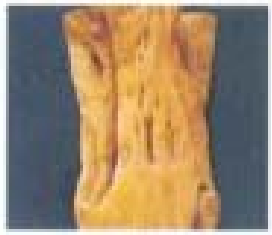
خسارت تریستزای مرکبات

زوال درختان مرکبات توسط ویروس تریستزا

علائم:

رایجترین علائم بیماری شامل توقف رشد، ضعف، زردی ، گلدهی بی موقع، زوال تدریجی یا سریع درختان آلوده روی پایه نارنج و سایر پایه های حساس است. این حالت در اثر تخریب آوندها و اختلال در رسیدن مواد غذایی به ریشه ایجاد میگردد.

با آغاز آلودگی ریشه های کوچکتر به تدریج پوسیده و قدرت جذب آب و مواد غذایی در درخت کاهش مییابد و در اثر انسداد آوندهای آبکش تجمع شیره پرورده در بخش بالایی گیاه افزایش یافته و باعث تولید بیش از حد گل و میوه میگردد. گاهی نشانه های دیگر نظیر نوارهای زرد یا قهوهای در محل پیوند و یا فرورفتگی ریز زیر پوست نارنج تحت عنوان علائم لانه زنبوری (Honey combing) یا ساقه آبله ای معکوس (Inverse stem pitting) را میتوان با برداشتن نواری از پوست در محل پیوند درختان آلوده مشاهده نمود.



علائم بیماری روی لیمو ترش (گیاه محک)

علائم ساقه آبلهای روی لیمو ترش

علائم لانه زنبوری معکوس در محل پیوند

برخی از نژادهای عامل بیماری ایجاد علائم ساقه آبله ای مینماید که در آن فرورفتگیها یا شیارهای ریز یا طویل در روی چوب سرشاخه ها، شاخه و تنه درختان آلوده به وجود میآید که با برداشتن پوست این قسمتها قابل رویت است.

چگونگی اپیدمی ویروس:

در شرایطی ویروس به حالت اپیدمی در میآید که:

1-نژاد ویروس حالت تهاجمی داشته باشد.

2- ناقل در منطقه موجود و دارای انتقال مؤثر باشد.

3 - ترکیب پایه و پیوندک (پایه نارنج و لیموترش حساس است).

کنترل:

1- رعایت مسائل قرنطینه ای

2- تهیه پیوندک از درختان سالم و عاری از ویروس و نظارت بر مراکز تولید نهال.

3- ریشه کنی درختان آلوده در شروع آلودگی.

4-استفاده از ترکیب پایه و پیوندک مناسب و متحمل به بیماری.

بیماری اگزوکورتیس مرکبات

اگزوکورتیس یکی از بیماریهای مهم ویروئیدی مرکبات درشمال ایران است. ارقام مرکبات پیوند شده روی پایه پونسیروس یا هیبریدهای آن مانند سیترنج حساس به بیماری میباشند.

عامل بیماری، ویروئید اگزوکورتیس مرکبات است. این ویروئید از طریق پیوندک آلوده و به صورت مکانیکی با ابزار باغبانی قابل انتقال میباشد.

علایم بیماری

در درختهای بیمار توقف رشد و شیارهای طولی و یا پوسته پوسته شدن نواری پوست تنه(Bark scalling)در قسمت پایه دیده میشود . این علائم ۳ تا ۸ سال پس از پیوند ارقام آلوده مرکبات روی پایه های حساس به ویژه پونسیروس (Poncirus trifoliata) و تعدادی از دو رگ های آن مانند سیترنج ها ایجاد میگردد.

برخی دیگر از ارقام نظیر لیمو شیرین، لمون و بالنگ نیز به این بیماری حساسند ولی شدت علائم تنه در آنها خفیف تر است. اگر ارقام آلوده به بیماری روی پایه های متحمل پیوند شوند، ممکن است حالت پا کوتاهی و کاهش رشد درخت مشاهده گردد. در هر صورت عامل بیماری در چنین ارقامی (حتی بدون وجود علائم) قادر به تکثیر بوده و میتواند به عنوان منابع آلودگی عمل نماید. شناسایی بیماری با ایندکس بر روی گیاهان محک امکانپذیر میباشد.

علائم پوسته پوسته شدن روی پایه (پونسیروس) در درخت مبتلا به اگزوکورتیس

کنترل

استفاده از پیوندک سالم، عدم استفاده از میزبانهای حساس و ضدعفونی ادوات باغبانی با هیپوکلریت سدیم.

بیماری کیسه صمغی

بیماری کیسه صمغی اولین بار در دهه ۱۹۳۰ بوسیله باغداری در کالیفرنیا مشاهده و توسط Fawcett, 1936 گزارش گردید. با واردات ارقام مرکبات از سال ۱۳۰۹ از کشورهای مختلف این بیماری وارد ایران شد.

عامل بیماری:

عامل بیماری احتمالا ویروس است و از طریق پیوندک آلوده به راحتی قابل انتقال است. بعضی گزارشها درباره انتقال بیماری با بذر و دانه گرده نیز وجود دارد.

علایم بیماری:

در درختان آلوده ارقام حساس(پرتقال تامسون ناول، نارنگی و تانجلو) وجود حفرات طولی با ابعاد مختلف روی تنه و شاخه ها همراه با ترشح صمغ و ضعف عمومی درخت به همراه خشکیدگی مشاهده میگردد و در وضعیت شدید زوال و مرگ درختان را موجب میگردد. در حالتیکه تعداد حفرات زیاد باشد باعث تغییر شکل تنه و شاخه ها میشود. در بعضی شرایط ترشح صمغ از سطح پوست در لبه یا وسط فرورفتگی یا شکافهای اطراف دیده میشود. روی برگهای جوان علایم نقش برگ بلوطی در شرایط خنک سال (بهار و پاییز) ظاهر میگردد که با گرم شدن هوا و کامل شدن برگها این علایم محو میشود.

فرورفتگیهای روی تنه در درختان آلوده

کنترل بیماری:

1- شناسایی و حذف درختان و نهال های آلوده مرکبات خصوصا رقم تامسون ناول با عارضه سرخشکیدگی و زوال با علایم مشخص آلودگی به بیماری کیسه صمغی

2- تهیه بذر و پیوندک از درختان مادری سالم جهت کنترل بیماری توصیه میشود.

جاروی جادوگر مرکبات

عامل بیماری: Candidatus phytoplasma aurantifolia

این بیماری اولین بار در اواخر دهه ۱۹۷۰ میلادی در کشور عمان مشاهده شد. این بیماری در حال حاضر در استانهای هرمزگان و سیستان و بلوچستان به شدت درختان لیمو ترش(لایم) را مورد حمله قرار داده است.

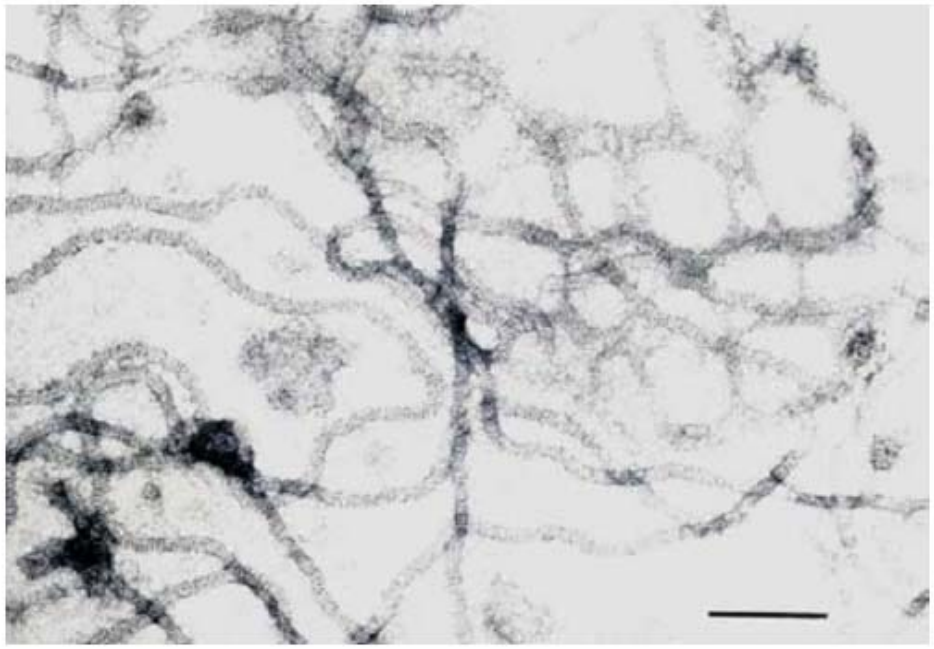
علایم بیماری:

در لایم آلوده فاصله میان گره ها کم شده و تعداد زیادی سرشاخه های ضعیف، متراکم و غیرطبیعی با برگهای ریز و رنگ پریده تولید میشود که حالت جارویی به سرشاخه آلوده میدهد. در شاخه های جارویی گل و میوه تشکیل نمیشود. با پیشرفت بیماری شاخه ها خشک شده و در نهایت منجر به مرگ درخت میشود. این بیماری توسط پیوندک آلوده و زنجرک منتقل میشود.

علایم سر خشکیدگی

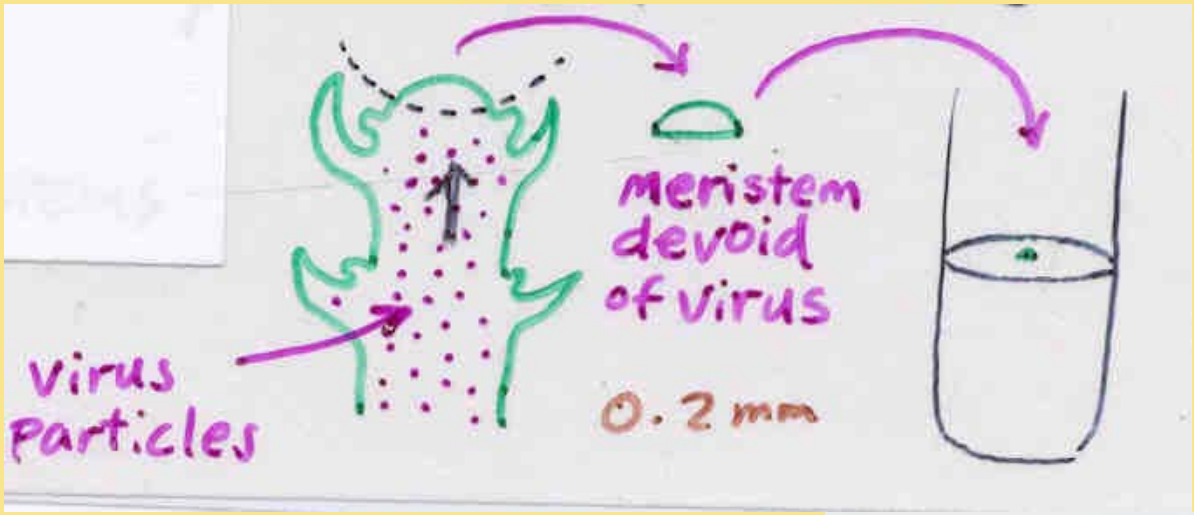
کنترل:

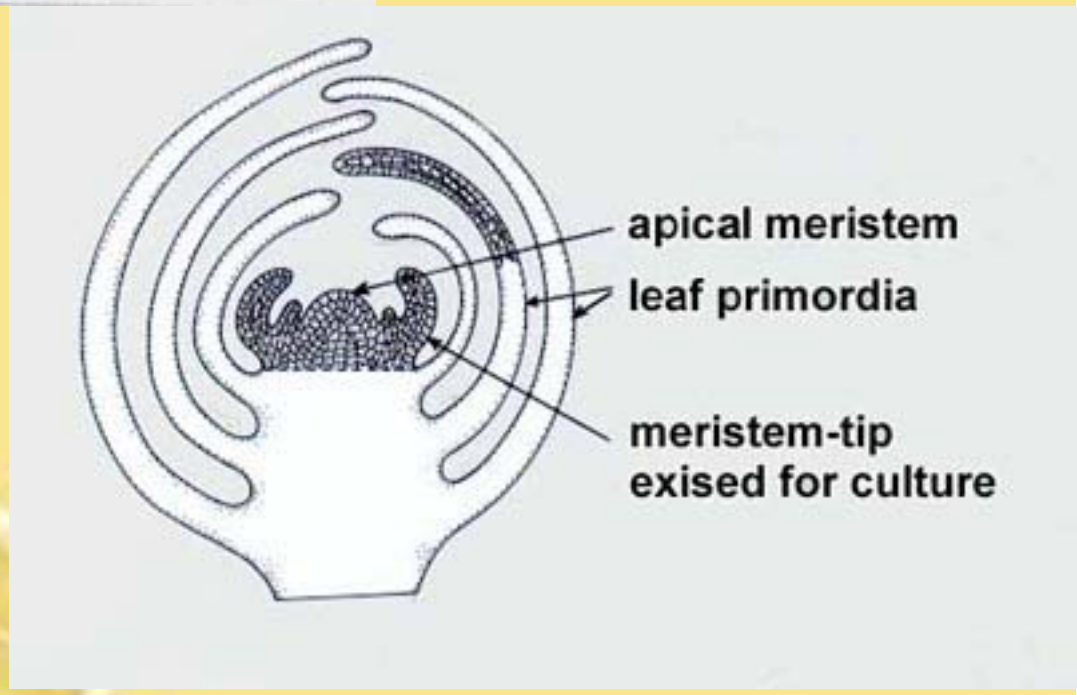
بهترین روش مبارزه با آن جلوگیری از ورود بیماری به مناطق سالم و ریشه کنی درختان آلوده میباشد. استفاده از ارقام مقاوم و مبارزه با حشرات ناقل نیز توصیه میشود



Electron microscopic picture showing Citrus tristeza closterovirus *particles. Bar represents 100 nm.*

Yellowing symptom on younger leaves of Eureka lemon seedlings caused by seedling yellows strain of CTV on left; healthy seedling on right.


meristem
devoid
of virus
0.2 mm
virus
particles


apical meristem
leaf primordia
meristem-tip
exised for culture

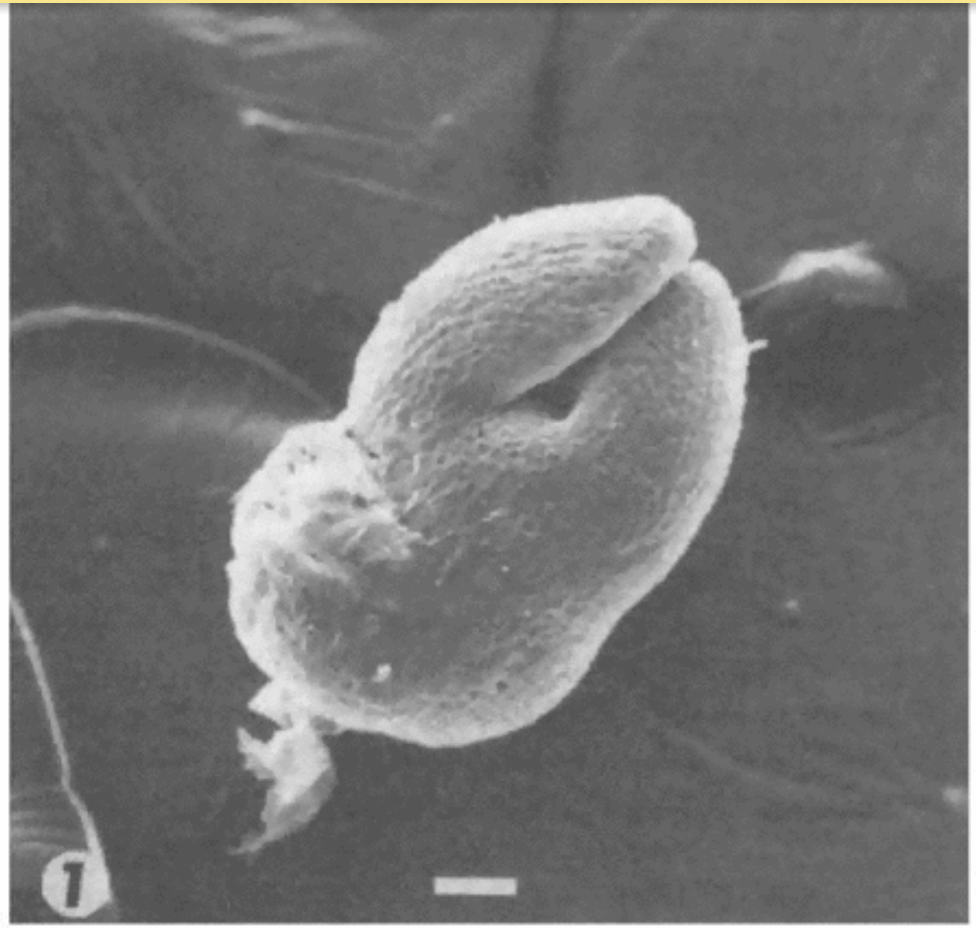


Fig. 1. A freshly excised meristem tip from an axillary bud of the potato *Solanum tuberosum*. The two smallest emergent leaf primordia are present. Scale bar represents 50 μ*M*.

Somatic embryogenesis from nucellar embryos in kinnow mandarin: (a) Embryogenic callus from nucellar embryos on MS medium supplemented with malt extract (400 mg L^{-1}) and 2,4-D (9.02 µM). (b) Somatic embryo formation on MS medium containing malt extract (500 mg L^{-1}) and ABA (7.56 µM). (c) Plantlet development from somatic embryos on ½ MS medium with NAA (10.74 µM). (d) A single plantlet on ½ MS medium with NAA (10.74 µM). (e) Nucellar embryo culture raised acclimatizated plantlet

ESTABLISHMENT OF VIRUS-FREE CITRUS NURSERY SYSTEM

- Shoot-tip micrografting (STG)
  - Preparation of rootstock seedling
  - Preparation of citrus shoot
  - Micrografting of shoot-tip
  - Double grafting

Preparation of rootstock seedling

Troyer of Carrizo citrange is the commonly used rootstock for STG. However, other citrus cultivars such us pummelo, lemon and sweet orange are also used.

(A) Culture of rootstock in solid medium.

[15]Preparation of Citrus STG Medium (pH 5.7)

1. Solid medium for growing rootstock seedling: MS salt mixture (Gibco BRL), 2.5 g; sucrose, 20 g; ddH_2O, 1 L; Agar, 1% (10 g)

2. Liquid medium for growing STG seedling: MS salt mixture, 2.5 g; sucrose, 30 g; growth factors (1 L/100X stock; i-inositol, 100 mg; thiamine-HCl, 0.2 mg; pyridoxine-HCl, 1 mg; nicotine acid, 1 mg); ddH_2O, 1 L

Preparation of citrus shoot

Young shoots are collected from infected citrus trees in orchards.
Alternatively potted citrus plants in green house are forced to sprout by defoliating and/or pruning. Young shoots of adequate size (0.5 cm~3 cm) are as scion collected for STG.

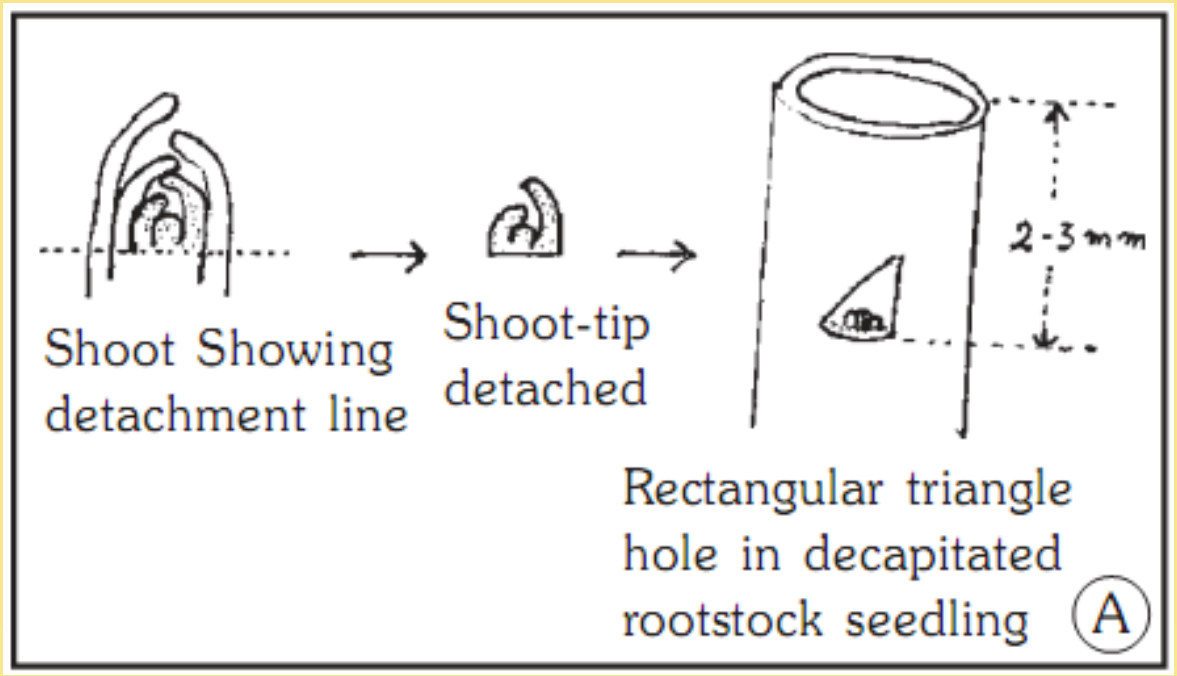
shoots of different sizes for STG.

Micrografting of shoot-tip

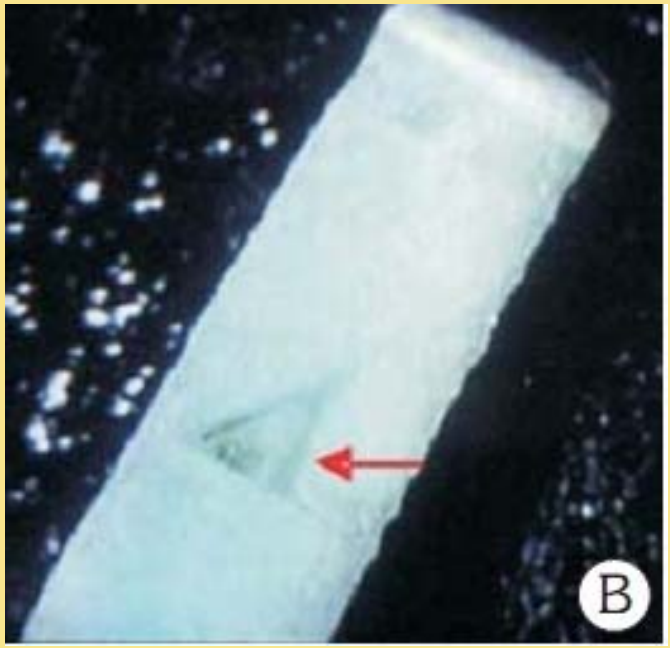
(C) Cutting knife consists of cutting edge of razor blade and edge holder; pliers for making blade edge.

(D) Making STG incision on upper top of rootstock seedling with tip-bended forceps and cutting knife.

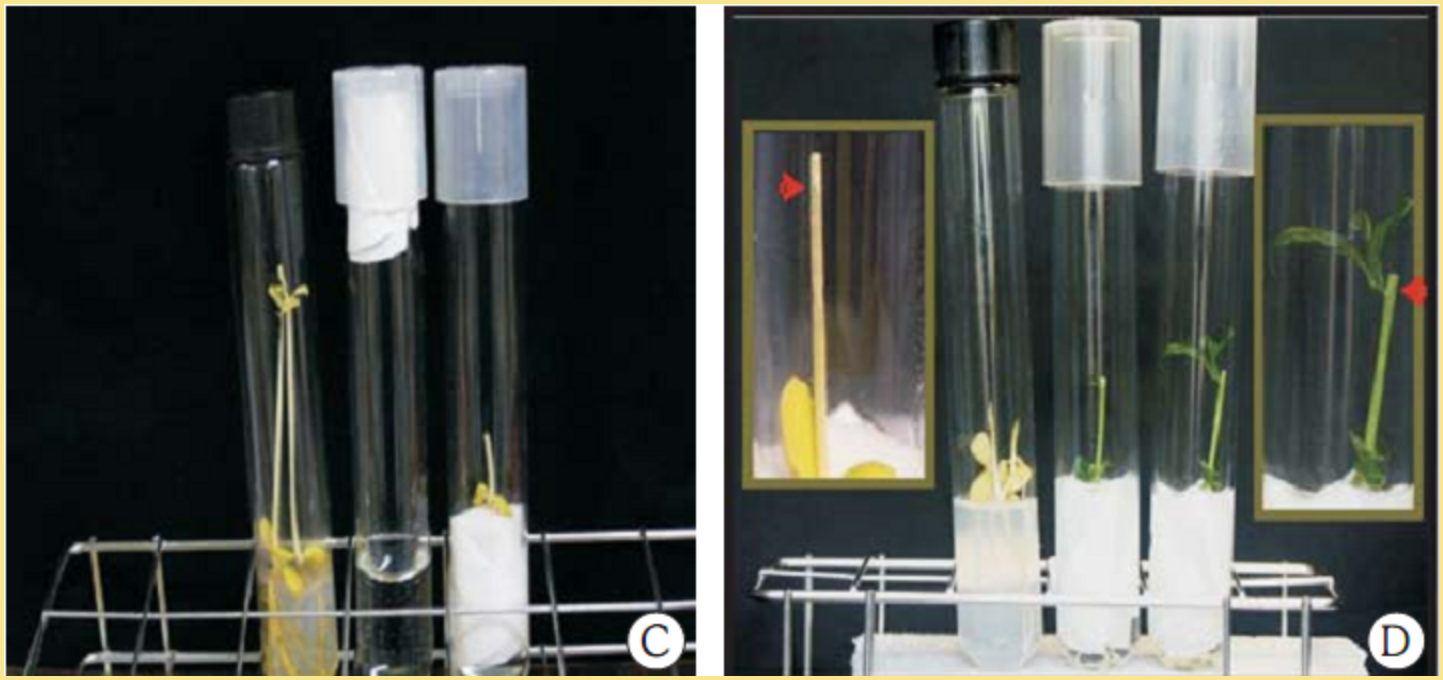
(E) Cold beam illuminator, binocular microscope and accessories within a laminar-flow bench. (F) Making STG incision on upper top of rootstock seedling with tip-bended forceps and cutting knife.



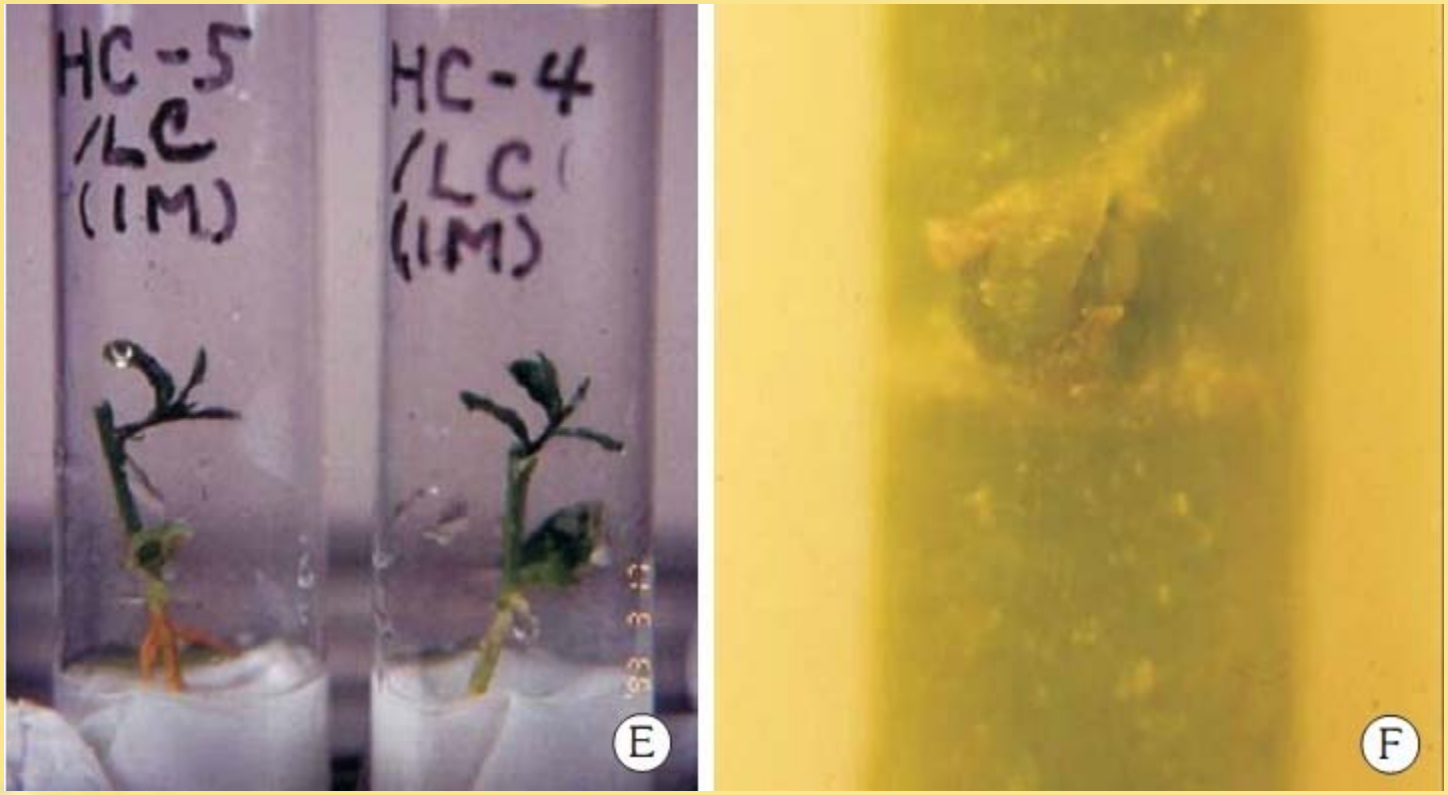
(A) Diagram showing excision of shoot-tip with 2-leaf primordia and rectangular triangle hole (0.3~0.5 mm) on a decapitated rootstock seedling by removing cortex layer with cutting edge of STG knife.



(B) A shoot tip placed in the hole on a decapitated rootstock seedling.



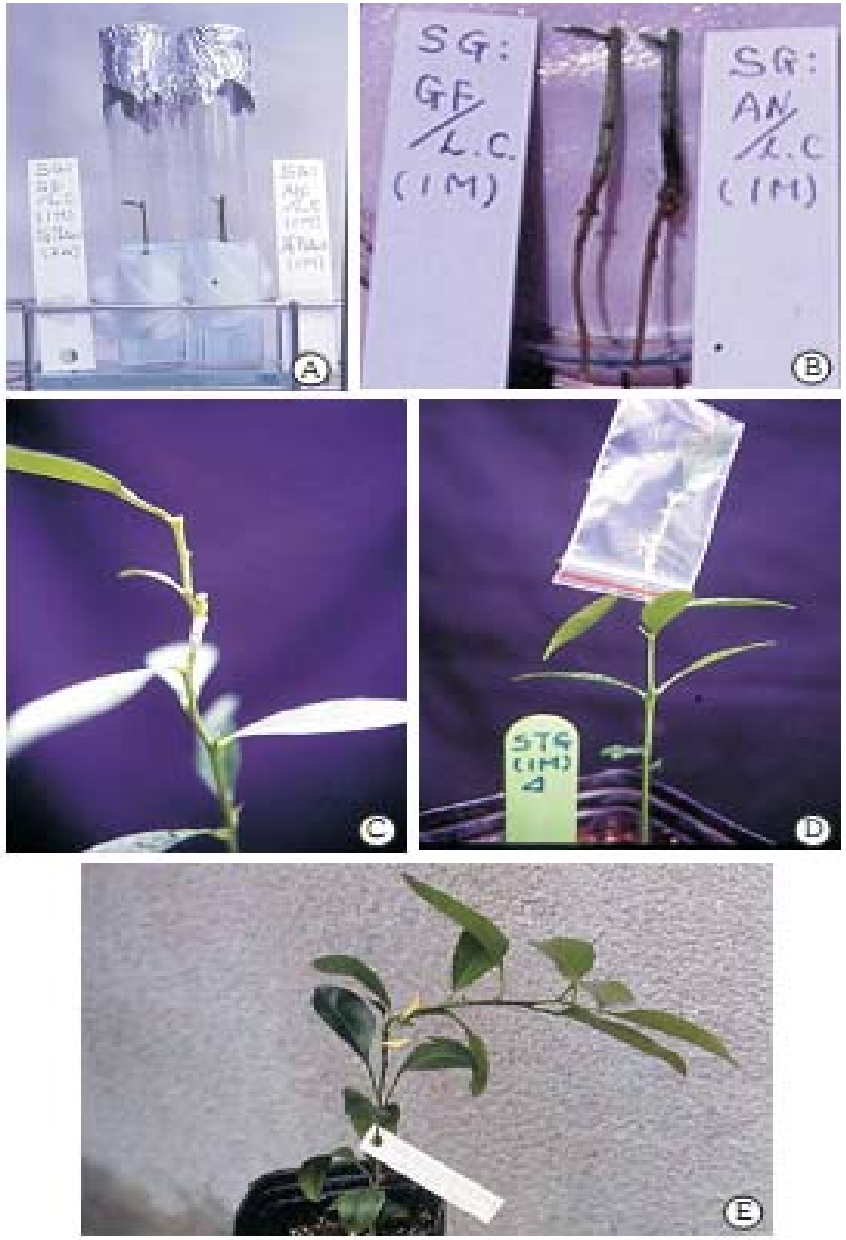
(C) Two-week old rootstock seedlings in solid medium (left), a sterile test tube with a center-perforated filter-paper platform containing liquid medium (center), and a test tube containing the micrografted rootstock seedling supported by filter paper platform on liquid medium (right). (D) Different stages of STG rootstock seedlings, also showing a new sprout regenerated from the grafted shoot-tip (right).



(E) Two new shoots of Hong China sweet orange regenerated from ST in rectangular hole (left) and in V-shaped incision (right) of rootstock seedlings one month after micrografting. (F) A new sprout from ST in rectangular hole on rootstock.

Double grafting

A double grafting technique has been developed to enhance the growth of STG-plants.



Procedure of double grafting with micrografted rootstock seedlings as scion. (A) Sprouting of micrografted seedlings in test-tube culture. (B) Two STG seedlings with sprouts taken out from test tubes for secondary grafting. (C) A potted vigorous rootstock seedling side-grafted with a scion from the STG-seedling. (D) The grafted part of rootstock seedling covered with a mouth-sealed plastic bug. (E) A new mature twig grown from the double grafted rootstock three months after double grafting.

Scientia Horticulturae 125 (2010) 361–367

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Micrografting of almond (*Prunus dulcis* Mill.) cultivars "Ferragnes" and "Ferraduel"

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ARTICLE INFO

Article history:
Received 9 December 2009
Received in revised form 21 March 2010
Accepted 13 April 2010

Keywords:
Almond
Micrografting
Restoring
Rejuvenation
Rootstocks
Scions

ABSTRACT

The success of various *in vitro* micrografting techniques, establishment of the rootstock, size of the microscion, and the effects of culture medium on the grafted seedling development for almond cultivars "Ferragnes" and "Ferraduel" were studied. *In vitro* germinated wild almond seedlings developed from seeds were used as rootstocks. Shoot culture initiation was successfully achieved from the above almond cultivars by culturing mature shoot tips from forced nodal buds, about 3–5 mm, on 0.7 mg/L BA and 0.01 mg/L NAA containing a MS medium. The regenerated adventitious shoots from *in vitro* cultures were maintained and proliferated by sub-culturing on a fresh medium every three to 4 weeks. Regenerated shoot tips, which were micrografted onto *in vitro* seedlings, resulted in the restoration of shoot proliferation. The results indicated that the most successful method for the grafting of tested almond cultivars was slit micrografting. High levels of micrograft take were achieved with all ranges of scions (4–15 mm) obtained from the regenerated shoot tips. Slow growth and lack of axillary shoot development on the micrografts were noticeable when the micrografts were cultured on hormone-free germination medium. *In vitro* micrografted plantlets were successfully acclimatized and no problems were encountered with the establishment of micrografted plants *in vivo*. The developed technique has demonstrated a high potential for application in the micropropagation of almond cvs. "Ferragnes" and "Ferraduel" and thereby, represents a feasible method for the renewal of almond orchards in Turkey and elsewhere in the world.

ELSEVIER

Scientia Horticulturae 92 (2002) 177–182

SCIENTIA HORTICULTURÆ

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Short communication

In vitro grafting of cashew (*Anacardium occidentale* L.)

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Accepted 11 May 2001

Abstract

A successful micrografting technique in cashew was developed using in vitro germinated seedlings as rootstocks and axenic shoot cultures (shoot-tip and nodal cultures) established from mature tree source as microscions. In vitro germinated seedlings, which emerged 20–25 days after inoculation on absorbent cotton, were decapitated and used as rootstock. Mature tree explants initiated on hormone-free Murashige and Skoog [Physiol. Plant. 15 (1962) 473] (MS) modified medium were made into scion of 3–15 mm length for grafting. Micrografts could be easily cultured on hormone-free liquid half-MS medium and were potted out after 10–12 weeks of culture growth. Grafting success was dependent on the method of grafting and size of the scion. Shoot-tip grafting and side grafting were equally successful (79.5–100%). Length of scion shoot had significant effect on micrografting success. Graft success was high (79.5%) when the scion length was >5 mm and it was less (0.5%) when size of scion was small (3–5 mm). Scion presoaked in either water or 0.01% ascorbic acid and 0.015% citric acid (1:1) reduced phenolic browning and drying of scion. Micrografting techniques standardized could be used for rejuvenation of shoot explants of mature tree.

INTRODUCTION

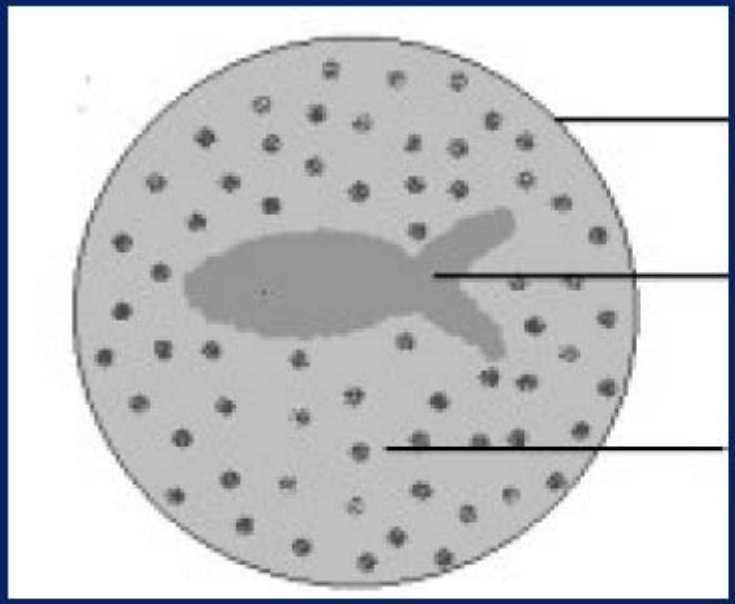
WHAT IS ARTIFICIAL SEED..?

ARTIFICIAL SEED CAN BE DEFINED AS ARTIFICIAL ENCAPSULATION OF SOMATIC EMBRYOS, SHOOT BUD OR AGGREGATES OF CELL OF ANY TISSUES WHICH HAS THE ABILITY TO FORM A PLANT IN IN-VITRO OR EX-VIVO CONDITION. ARTIFICIAL SEED HAVE ALSO BEEN OFTEN REFERRED TO AS SYNTHETIC SEED.

ARTIFICIAL SEEDS

Concepts -

- Artificial seeds were first introduced in 1970's as a novel analogue to the plant seeds. The production of artificial seeds is useful for plants which do not produce viable seeds. It represents a method to propagate these plants. Artificial seeds are small sized and these provides further advantages in storage, handling and shipping.
- The term, "EMBLING" is used for the plants originated from synthetic seed.
- The use of synthetic varieties for commercial cultivation was first suggested in Maize (Hays & Garber, 1919).



THE CONCEPT OF ARTIFICIAL SEED

UTILIZATION OF ARTIFICIAL SEEDS

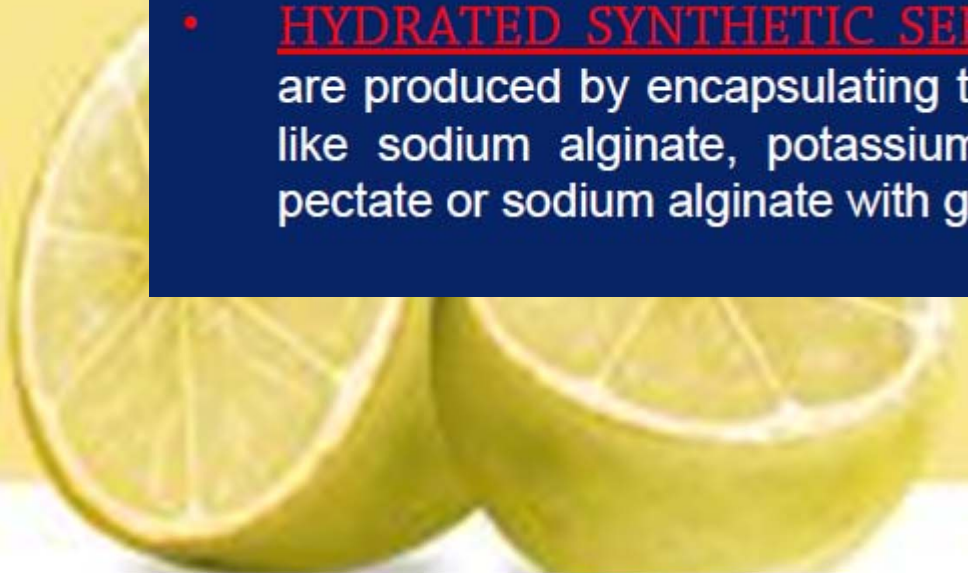
- The artificial seeds can be used for specific purposes, notably multiplication of non-seed producing plants, ornamental hybrids or the propagation of polyploid plants with elite traits.
- It can be employed in the propagation of male or female sterile plants for hybrid seed production.
- Cryo-preserved artificial seeds may also be used for germplasm preservation particularly in recalcitrant species (*such as mango, cocoa and coconut*), as these seed will not undergo desiccation.

- Transgenic plants, which require separate growth facilities to maintain original genotypes may also be preserved using somatic embryos.
- Somatic embryogenesis is a potential tool in the genetic engineering of the plants.
- Plants that are generated by somatic embryos from single transgenic cell, the progeny will not be chimeric.

- Multiplication of elite plants selected in plant breeding programs via somatic embryos avoids the genetic recombination, and therefore does not warrant continued selection inherent in conventional plant breeding, saving considerable amount of time and other resources.
- Artificial seeds produced in tissue culture are free of pathogens. Thus, another advantage is the transport of pathogen free propagules across the international borders avoiding bulk transportation of plants, quarantine and spread of diseases.

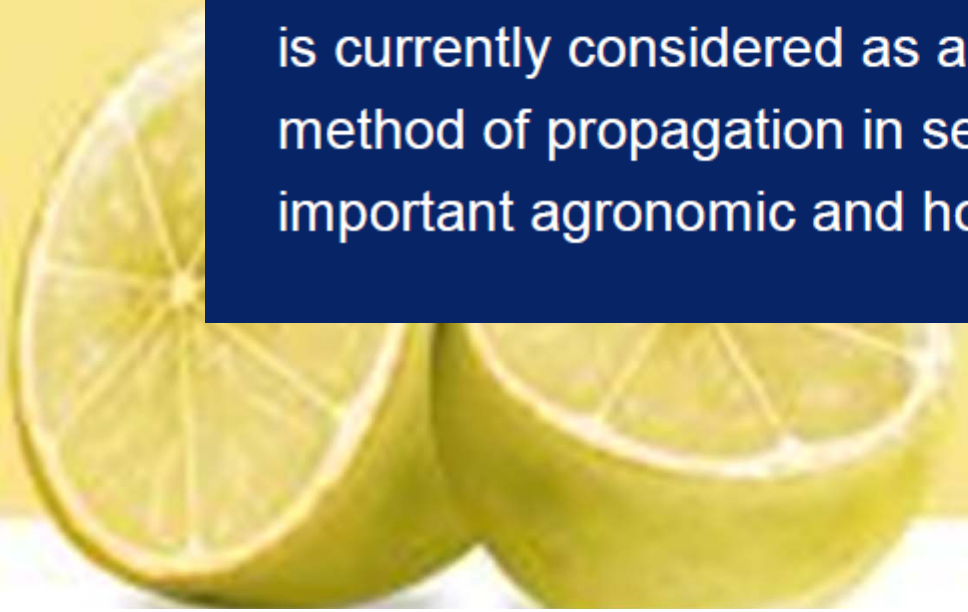
BASED ON THE TECHNIQUES TWO TYPES OF ARTIFICIAL SEEDS ARE PRODUCED

- DESICCATED SYNTHETIC SEEDS- Desiccated synthetic seeds are produced nacked or polyoxyethylene glycol encapsulated somatic embryos. This type of synthetic seeds is produced in desiccation tolerant species plant.
- HYDRATED SYNTHETIC SEEDS- Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogels like sodium alginate, potassium alginate, carrageenan, sodium pectate or sodium alginate with gelatine.



NEED FOR ARTIFICIAL PRODUCTION TECHNOLOGY

- Development of micro propagation technique will ensure abundant supply of desired plant species.
- Development of artificial seed production technology is currently considered as an effective and efficient method of propagation in several commercially important agronomic and horticultural crops.



- These artificial seed would also be a channel for new plant lines produced through biotechnological advances to be delivered directly to greenhouse and field.
- High volume propagation potential of somatic embryos combined with formation of synthetic seeds for low-cost delivery would open new vistas for clonal propagation in several commercially important crop species.

WHAT ARE SOMATIC EMBRYOS ?

Somatic embryos are bipolar structures with both apical and basal meristematic regions, which are capable of forming shoot and root respectively.

SOMATIC EMBRYOS vs ZYGOTIC EMBRYOS AND THEIR ADVANTAGES.

- Somatic embryos are structurally similar to zygotic embryos found in seeds and possess many of their useful features, including the ability to grow into complete plant.
- Somatic embryos differ in that they develop from somatic cells, instead of zygotes and thus, potentially can be used to produces duplicates of single genotypes.

- Somatic embryos develops from somatic cells(non-sexual) and does not involve sexual recombination. This characteristic of somatic embryos allows not only clonal propagation but also specific and directed changes to be introduced into desirable elite individuals by inserting isolated gene sequences into somatic cells.
- If the production efficiency and convenience comparable to that of a true seed are achieved, somatic embryos can be potentially used as a clonal propagation system.

BASIC REQUIREMENT FOR THE PRODUCTION OF ARTIFICIAL SEEDS.

- One pre-requisite for the application of synthetic seed technology in micropropagation is the production of high quality, vigorous somatic embryos that can produce plants with frequencies comparable to natural seeds.
- Synthetic seed technology requires the inexpensive production of large numbers of high quality somatic embryos with synchronous maturation.

- Encapsulation and coating systems, though important for delivery of somatic embryos, are not the limiting factors for the development of synthetic seeds.
- The lack of synchrony of somatic embryos is, arguably, the single most important hurdle to be overcome before advances leading to wide spread commercialization of synthetic seeds can occur.

PROCEDURE FOR PRODUCTION OF ARTIFICIAL SEEDS

Establish somatic embryogenesis

↓

Mature somatic embryogenesis

↓

Synchronize and singulate somatic embryos

↓

Mass production of somatic embryos

↓

Standardization of encapsulation

↓

Standardization of artificial endosperm

↓

Mass production of artificial seeds

↓

Greenhouse and field planting

Methods for artificial seed encapsulation

- **Dropping method**

  Somatic embryos are dipped in hydrogel, this step encapsulate SEs.

  Hydrogel used may be any of the following.

  - alginate – sodium alginate, agar from see weeds, seed gums like guar gum, locust bean gum.

  - Sodium alginate solution (1 – 5%), prepared in MS basal medium solution.

  - SEs are dipped in this solution.

  -These coated beads are added one by one into a complexation solution flask kept on magnetic stirrer and kept such for around 20-30 minutes.

- Embryos get covered by calcium alginate which is a stable complex due to ionic bond formation, become harder, Seeds become harder.
- Then gelled embryos are washed with water or MS basal medium.
- The synthetic seeds are ready.

Molding method

- This method follows simple procedure of mixing of embryos with temperature dependent gel (e.g. gel rite, agar).
- Cells get coated with the gel at lowering of the temperature.

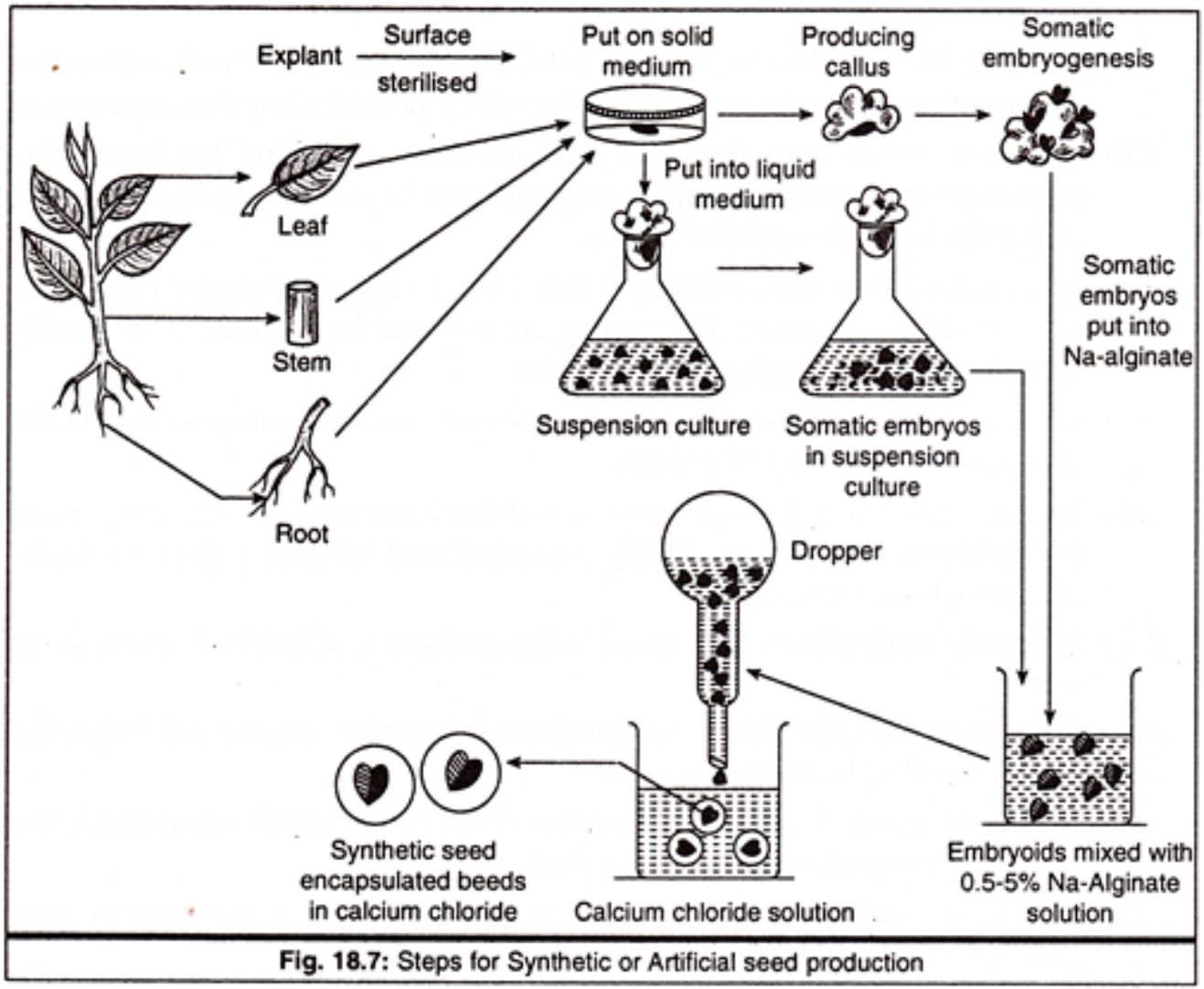
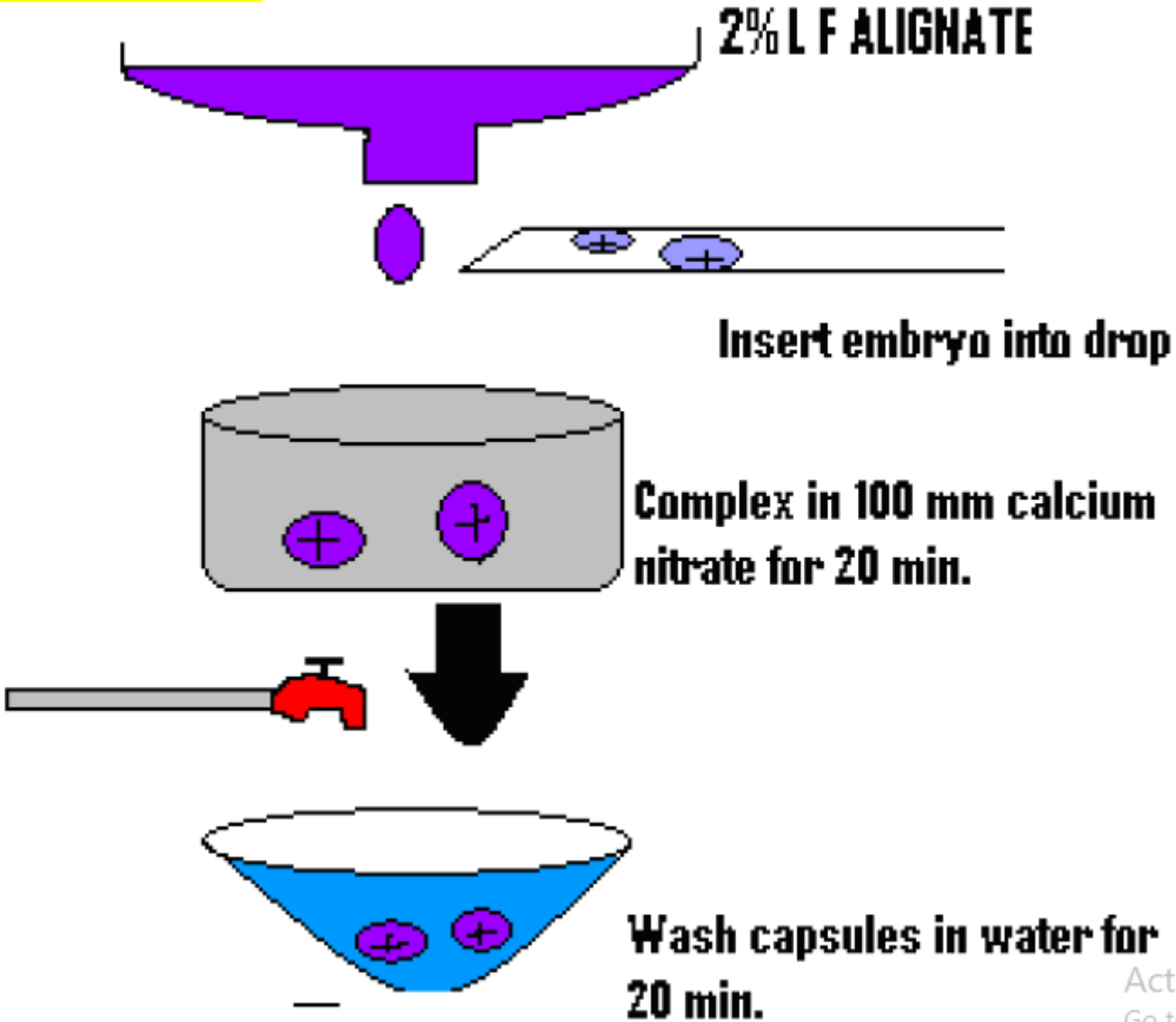


Fig. 18.7: Steps for Synthetic or Artificial seed production

ENCAPSULATION PROCESS



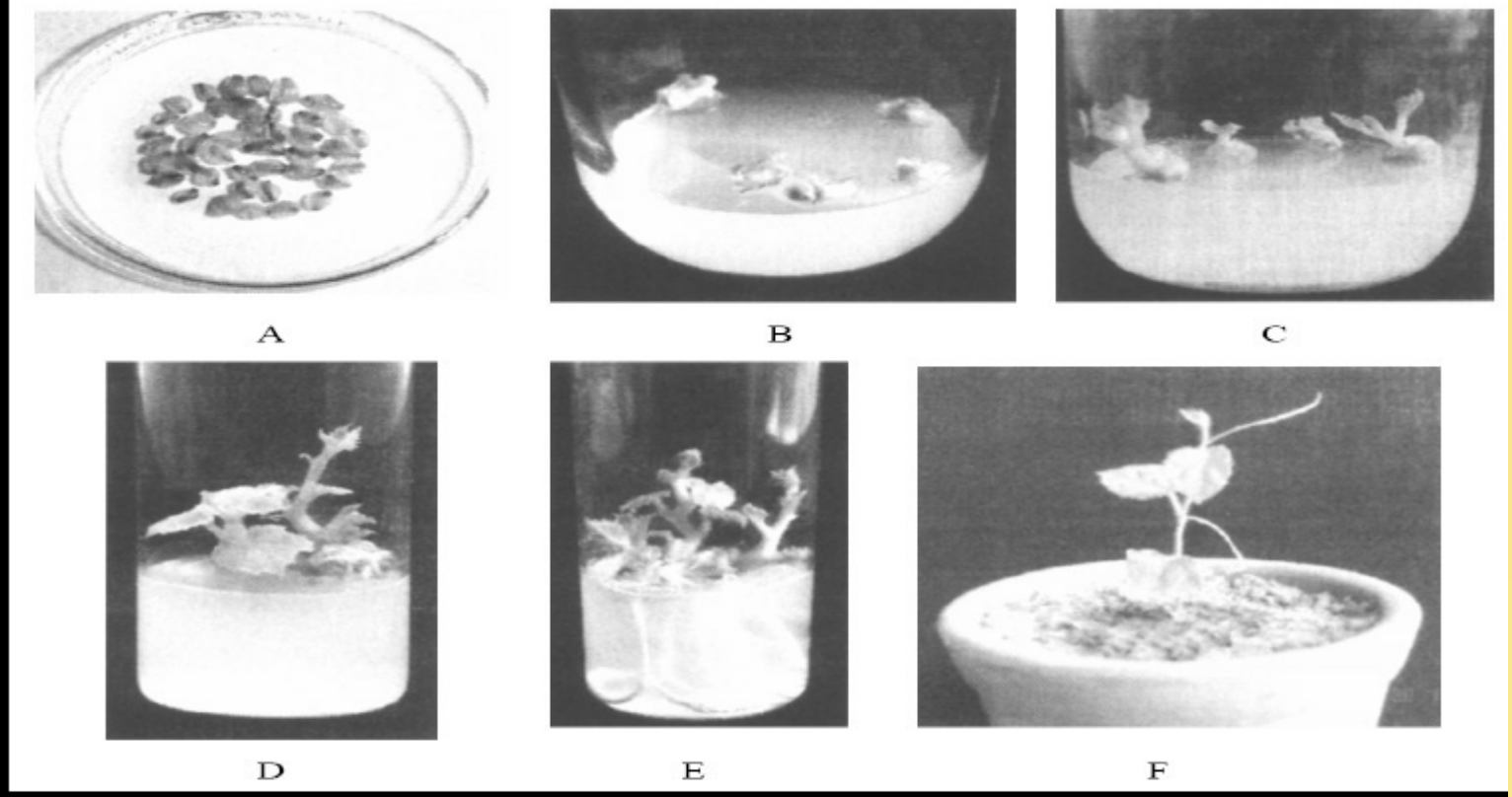
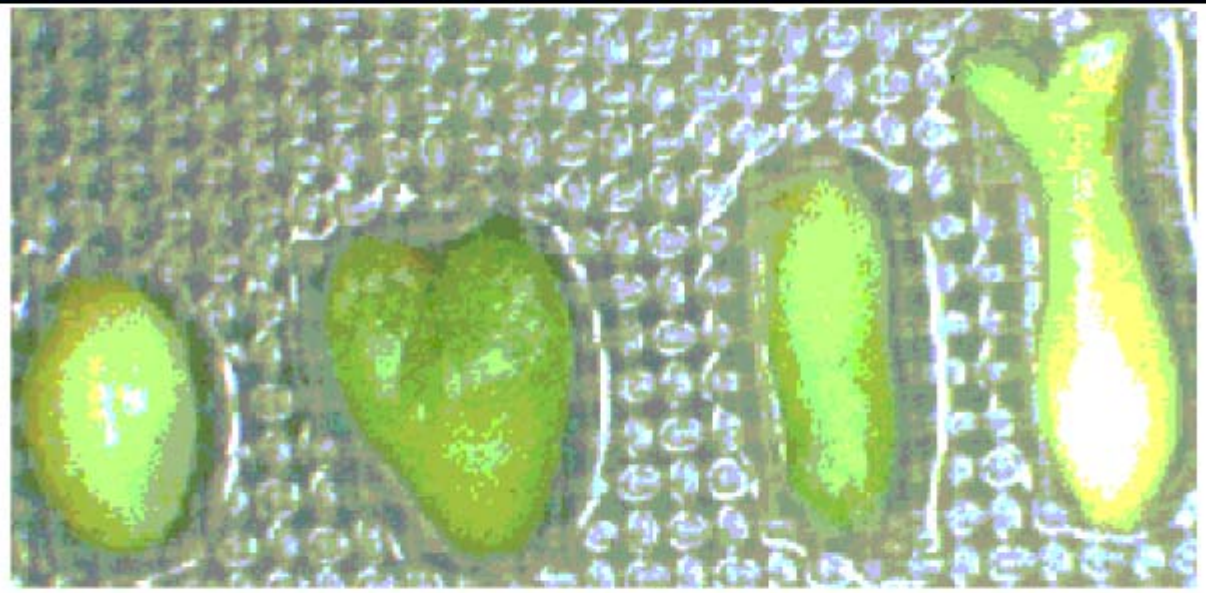


Fig: Regeneration of encapsulated shoot tips (artificial seed) of pointed gourd.

A. Encapsulated shoot tips in sodium alginate bead.
B. Germination of encapsulated shoot tips.
C. Shoot induction of encapsulated shoot tips.
D. Shoots from encapsulated shoot tips
E. Rooted multiple shoots of encapsulated shoot tips.
F. Established plant in earthen pot.

THE MORPHOLOGICAL STAGES OF SOMATIC EMBRYO DEVELOPMENT IN ALFA ALFA (Medicago sativa L.)



globular heart torpedo cotyledonary

DESICCATION TOLERANCE:

- ✓ Desiccation tolerance is a quantitative characteristic not a qualitative one.
- ✓ It can be induced by a pretreatment with ABA or stress to elicit the desired response.
- ✓ The type of pretreatment used, the duration for which it is applied and the stage of embryo that is treated are critical factors.
- ✓ For 3 days in 20 mm ABA is sufficient to induce tolerance, but chilling requires almost 3 weeks.

PRINCIPLE AND CONDITIONS FOR ENCAPSULATION WITH ALGINATE MATRIX

- The major principle involved in alginate encapsulation process is that the sodium alginate droplets containing the somatic embryos when dropped into $CaCl_2.2H_2O$ solution form round and firm beads due to ion exchange between the NA^+ in sodium alginate with CA^{2+} in the $CaCl_2.2H_2O$ solution.
- 3% sodium alginate upon complexation with 75mM $CaCl_2.2H_2O$ for half an hour gives optimum beads hardness and rigidity for the production of viable synthetic seeds.

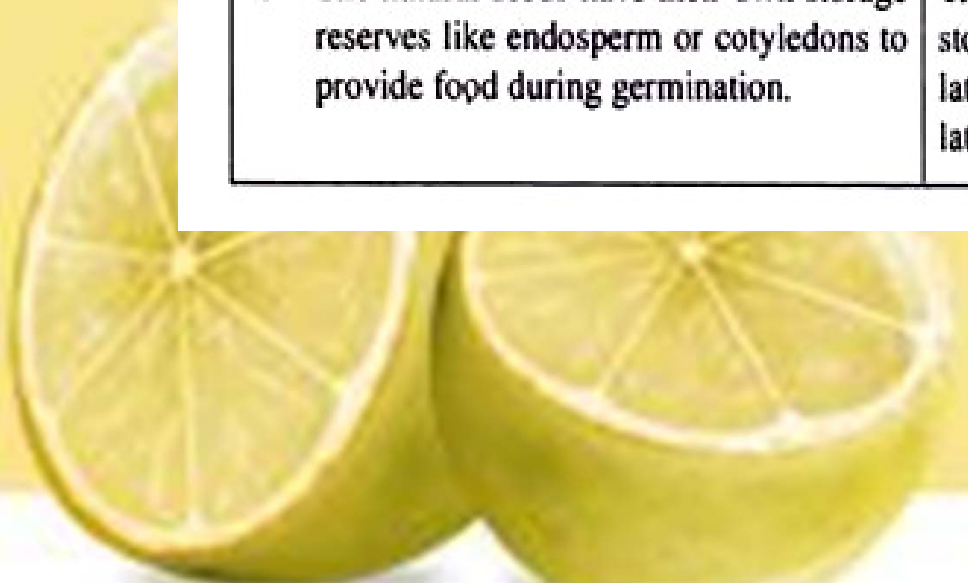
ADDITION OF ADJUVANTS TO THE MATRIX

- To prevent the embryo from desiccation and mechanical injury, a number of useful materials such as nutrients, fungicides, pesticides, antibiotics and microorganisms (eg.rhizobia) may be incorporated into the encapsulation matrix.
- Incorporation of activated charcoal improves the conversion and vigour of the encapsulated somatic embryos and retains nutrients within the hydrogel capsule and slowly releases them to the growing embryo.

ARTIFICIAL ENDOSPERM

- Somatic embryos lack seed coat (testa) and endosperm that provide protection and nutrition for zygotic embryos in developing seeds.
- To augment these deficiencies, addition of nutrients and growth regulators to the encapsulation matrix is desired, which serves as an artificial endosperm.
- These addition results in increase efficiency of germination and viability of encapsulated somatic embryos.these synthetic seeds can be stored for a longer period of time even upto 6 months without losing viabilty,especially when stored at 4^0c.

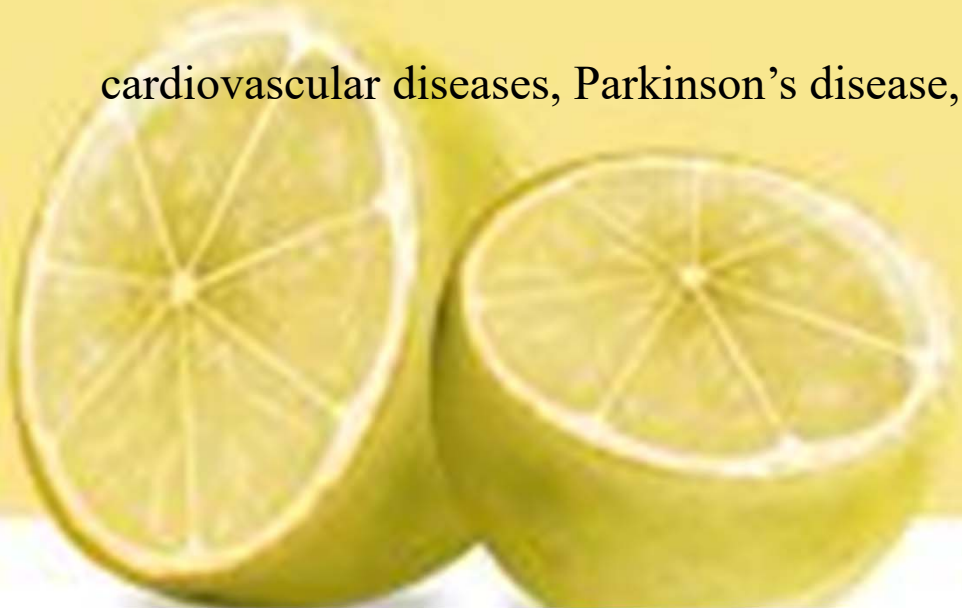
	Natural seeds	Synthetic seeds
1.	Hard seed coat present.	No seed coat, only encapsulated.
2.	Embryos are much protected within cotyledons or endosperm.	Embryos are not protected within any kind of maternal tissue.
3.	Embryos undergo controlled desiccation by the maternal tissue and have a natural dormancy period.	Embryos do not pass through any kind of desiccation and they the do not have any dormancy period.
4.	The natural seeds have their own storage reserves like endosperm or cotyledons to provide food during germination.	The artificial seeds do not have their own storage tissue, the nutrients or growth regulators can be supplied within the encapsulating material.



Regulation of Human Health Nutrients

Introduction

During metabolic process and contact process with external environment, a large amount of free radicals are produced in human body (Li et al., 2014). Overproduction of free radicals results in oxidative stress, a deleterious process that can be an important intervener of damage to cell structures, including proteins, fatty acids and nucleic acids (Valko et al., 2007). Thus, oxidative stress has been implicated in ageing and in a number of human diseases such as cancers, arteriosclerosis, diabetes, cataract, cardiovascular diseases, Parkinson's disease, Alzheimer's disease and arthritis.



Consumers see the connection between fruits and health and associate their diets with the prevention of these diseases. Numerous epidemiological and prospective studies have been undertaken that suggest a strong link between dietary intake of phytochemicals and human health, particularly in protecting against chronic degenerative diseases, such as cardiovascular disease, diabetes mellitus and cancers (Hajiaghaalipour et al., 2015). As the public becomes more aware of the health benefits of fruits, the demand for fruits specifically developed for their health benefits is increasing (Byrne, 2012). Such health-enhanced products that could be sold fresh or processed into extracts are natural sources of antioxidants, antimicrobials, or food colorants for the health and foot industries (Byrne, 2002; Cevallos-Casals et al., 2006).

Terms

Phytonutrient

A diet rich in fruits and vegetables provides an abundance of human health compounds. These compounds synthesized originally by plants or accumulated by plants and which are known to have a multitude of human health (wellness) benefits are called phytonutrients.

Biofortification

Biofortification is an effective and economical method to improve the micronutrient content of crops, particularly staples that sustain human populations in developing countries. Whereas conventional fortification requires artificial additives, biofortification involves the synthesis or accumulation of nutrients by plants at source.

biostimulants

Plant biostimulants, sometimes referred to as agricultural biostimulants, are a diverse classification of substances that can be added to the environment around a plant and have positive effects on plant growth and nutrition, but also on abiotic and biotic stress tolerance.

Functional foods

Using practical and new approaches to provide additional health benefits to consumers or animals by promoting the state of well-being and possibly reducing the risk of disease is called functional foods.

Nutraceutical

When functional food aids in the prevention and/or treatment of disease(s) and/or disorder(s) other than anemia, it is called nutraceutical.

Box 1 Glossary.

- *Supplementation* is the oral delivery of micronutrients in the form of pills, or powdered formulations that are dissolved before administration [3].
- *Fortification* is the practice of deliberately increasing the content of an essential micronutrient and thus improving the nutritional quality of food, for example, iron and zinc added to flour, and iodine added to table salt [3,61].
- *Biofortification* is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology [61].
- *Bioaccessibility* is the amount of an ingested nutrient that is released from the food matrix in the gastrointestinal tract and becomes available for absorption [23*,62].
- *Bioavailability* is the amount of an ingested nutrient that is available for utilization or for storage, including gastrointestinal digestion, absorption, metabolism, tissue distribution, and bioactivity [23*,62].

biofortification

Strategies to address micronutrient deficiency include dietary diversification, nutritiona supplements, fortification and biofortification. A combination of approaches is likely to provide the greatest overall benefit, but in some populations **dietary diversification** is impractical and supplements are only suitable as shortterm interventions. **Fortificatior** requires the addition of nutrients to food products, for example, iodine is added to table salt, and iron, zinc and folate are added to flour to make bread.

One major drawback of these approaches is the limited stability of the additives, fo example, folate added to rice becomes more soluble at higher temperatures and is los when the rice is boiled.

A second disadvantage is that additives can also affect the quality of food, for example iron additives are oxidized over time and this has an impact on taste.

The third and major limitation of conventional fortification is that it is mainly suited to developed countries with the necessary technical infrastructure and distributior networks, but is less appropriate for developing countries with their extensive reliance or subsistence agriculture.

biostimulants

The industry definition of biostimulants was originally proposed in 2012 and stated: “Plant biostimulants contain substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and **crop quality.**

Biostimulants:

- protein hydrolysates
- seaweed extracts
- silicon
- chitosan
- Humic and fulvic acids phosphite
- Arbuscular mycorrhizal fungi *trichoderma*
- Plant growth-promoting rhizobacteria

biostimulants ameliorates the effects of abiotic stress (Table 1).

A. brasilense	*L. sativa*	Salt tolerance	[19, 20]
A. brasilense	*T. aestivum*	Salt and osmotic stress	[21]
A. brasilense	*L. lycopersicum*	Drought tolerance	[22]
A. brasilense/P. dispersa	*C. annuum*	Salt tolerance	[23]
A. chrococcum	*Z. mays*	Salt tolerance	[24]
A. chrococcum	*T. aestivum*	Salt tolerance	[25]
A. chrococcum	*T. aestivum*	Temperature tolerance	[26, 27]
A. lipoferum	*T. aestivum*	Salt tolerance	[28]
A. nodosum	*Kappaphycus alvarezii*	Cold tolerance	[29]
A. nodosum	*P. dulcis*	Ion homeostasis	[30]
A. nodosum	*C. sinensis*	Drought tolerance	[31]
B. phytofirman,	*Vitis vinifera*	Cold tolerance	[32, 33]
F. glaciei	*Solanum lycopersicum*	Cold tolerance	[34]
Fulvic and humic acids	*F. arundinacea*	Drought tolerance	[35, 36]
Fulvic and humic acids	*A. palustris*	Drought tolerance	[37]
Glycinebetaine	*L. lycopersicum*	Chilling stress	[38]
H. diazotrophicus	*H. vulgare*	Salt tolerance	[39]
Humic acid and phosporous	*C. annuum*	Salt tolerance and ion homeostasis	[40]
Humic acids	*O. sativa*	Oxidative and drought stress	[41]
Humic acids	*P. vulgaris*	Salt tolerance	[42]
Megafol	*L. lycopersicum*	Drought tolerance	[43]
Melatonin	*Z. mays*	Chilling tolerance	[44]
P. frederiksbergensis	*Solanum lycopersicum*	Cold tolerance	[34]
P. putida	*T. aestivum*	Heat tolerance	[45]
P. putida	*S. bicolor*	Heat tolerance	[46]
P. vancouverensis	*Solanum lycopersicum*	Cold tolerance	[34]
P.dispersa	*T. aestivum*	Cold tolerance	[47]
Protein hydrolysates	*H. vulgare*	Ion homeostasis	[48]
Protein hydrolysates	*Z. mays*	Salt tolerance	[49]
Protein hydrolysates	*T. aestivum*	Heavy metal tolerance	[50]
Protein hydrolysates	*L. sativa*	Salt tolerance, cold tolerance	[51, 52]
Protein hydrolysates	*D. kaki/D. lotus*	Salt tolerance	[53]
Protein hydrolysates	*Lolium perenne*	Heat tolerance	[51]
R. leguminosarum	*V. faba*	Salt tolerance	[54]
R. leguminosarum	*P. sativum*	Salt tolerance	[54]
SWE	*A. thaliana*	Cold tolerance	[55, 56]
SWE	*P. pratensis*	Salt tolerance	[57]
SWE	*A. stolonifera*	Heat tolerance	[58]
SWE	*S. oleracea*	Drought tolerance	[59]
SWE	*L. sativa*	Ion homeostasis	[60]

Seaweed extracts (SWE) as biostimulants are emerging as commercial formulations for use as plant growth promoting factors and a method to improve tolerance to salinity, heat, and drought. Algal extracts target a number of pathways to increase tolerance under stress (Fig. 1).

Seaweeds are red, green, and brown macroalgae that represent 10% of marine productivity [8].

Macroalgae have been used as organic fertilizers for thousands of years and are still in use [64].

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effects of a biostimulant derived from the brown seaweed *Ascophyllum nodosum* on ripening dynamics and fruit quality of grapevines

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ARTICLE INFO

Keywords:
Ascophyllum nodosum
Vitis vinifera
Seaweed extract
Anthocyanins
Phenolic maturity
Viticulture

ABSTRACT

Most modern and traditional grape-growing regions are facing challenging times due to the unpredictability of weather conditions and warming trends. Innovative and sustainable tools such as seaweed-based biostimulants may play a key-role in the development of environment-friendly viticultural strategies to improve yields, biotic/abiotic stress tolerance and fruit and wine quality. A sprayable *Ascophyllum nodosum* extract was tested on grapevines cv. Sangiovese grown under Mediterranean conditions (central Italy) and on grapevines cv. Pinot Noir and Cabernet Franc within a cool-climate viticulture region (Michigan, USA). The product was sprayed on the canopies at label doses (1.5 kg/ha) five times during the season, starting two weeks before veraison. The seaweed extract did not affect leaf gas exchanges, yield or cluster and berry size, but hastened veraison, improved anthocyanins accumulation in all cultivars and increased phenolic content particularly in Sangiovese. Therefore, medium-late application of the seaweed extract can be a simple way to favour chromatic and chemical proprieties of grapes and wines. This is the first report of positive effects of *Ascophyllum nodosum* extracts on the quality of cultivated wine grapes. The adoption of the technique can be particularly suitable to cool-climate viticulture, especially as it pertains to short growing seasons and genotypes with a limited phenolic profile.

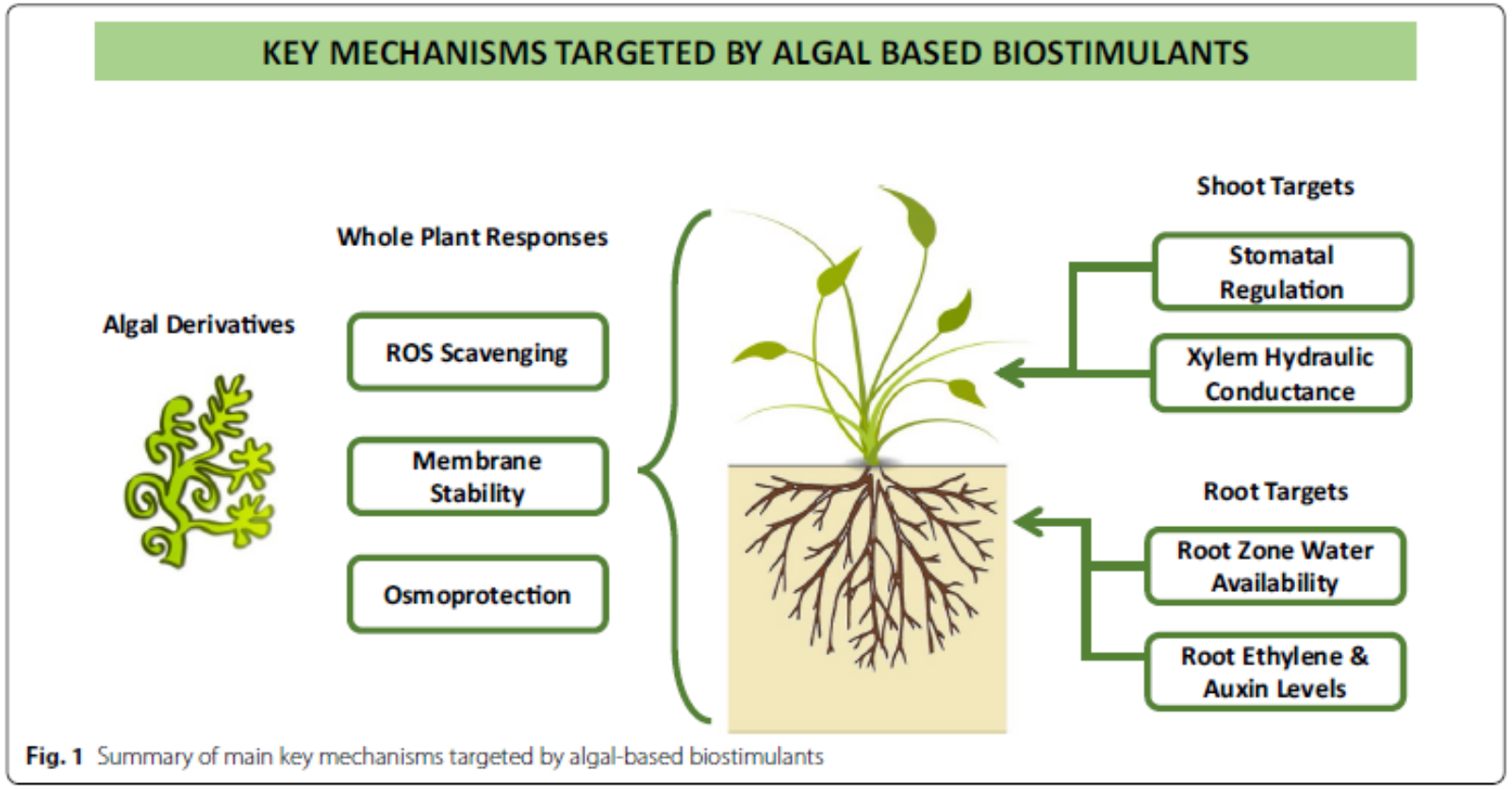

Fig. 1 Summary of main key mechanisms targeted by algal-based biostimulants

Protein hydrolysates are mixtures of polypeptides, oligopeptides, and free amino acids derived from partial hydrolysis of agricultural by-products from animals and plants. Carbohydrates, proteins, amino acids, and lipids may increase stress tolerance through different (Fig. 2).

The effects of amino acids on ion fluxes across membranes have been clearly established, with most having a positive effect on reducing NaCl-induced potassium efflux.

Protein hydrolysates (PH) are often sold as formulations that include plant growth regulators. The bulk of PH products, over 90%, are produced from chemical hydrolysis of animal by-products while enzymatically processed plant-based products are a recent development.

Scientia Horticulturae

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Research Paper

Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars

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ARTICLE INFO

Keywords:
Antioxidant activity
Lycopene
Mineral composition
Plant biostimulants
Solanum lycopersicum L.
Sustainable horticulture

ABSTRACT

The use of natural plant biostimulants is proposed as a promising and innovative approach to ensure improved and sustainable yields and product quality. A greenhouse experiment was performed to assess the yield performance, leaf net assimilation of CO_2, mineral composition of leaves and fruits, and fruit physicochemical quality attributes of two tomato cultivars (Akyra and Sir Elyan) in relation to biostimulant treatments (control or two different concentrations of the legume-derived protein hydrolysate Trainer®). Treated tomato plants were sprayed every 10 days with a solution containing 2.5 and 5.0 ml L^{-1} of biostimulant. Akyra was found to be richest in K, Ca, Mg, lipophilic and hydrophilic antioxidant activities (LAA and HAA), lycopene, total phenolic and total ascorbic acid. Foliar applications of legume-derived protein hydrolysate at 5.0 ml L^{-1} increased marketable yield of Akyra and Sir Elyan by modulating yield components differently depending on cultivars: higher number of fruits in Akyra and increase of fruit mean weight in Sir Elyan. Improved yield performance with biostimulant foliar applications at the highest rate was related to improved leaf nutritional status (higher K and Mg) and higher net assimilation of CO_2. The application of legume-derived protein hydrolysate at 5.0 ml L^{-1}, and to a lesser degree at 2.5 ml L^{-1}, elicited an increase in antioxidant activities, total soluble solids, mineral composition (K and Mg) as well as bioactive molecules such as lycopene and ascorbic acid, thereby increasing the nutritional and functional quality of the fruits. These findings can assist tomato growers in selecting cultivars and application dose for protein hydrolysate to complement high crop productivity with optimal fruit quality.

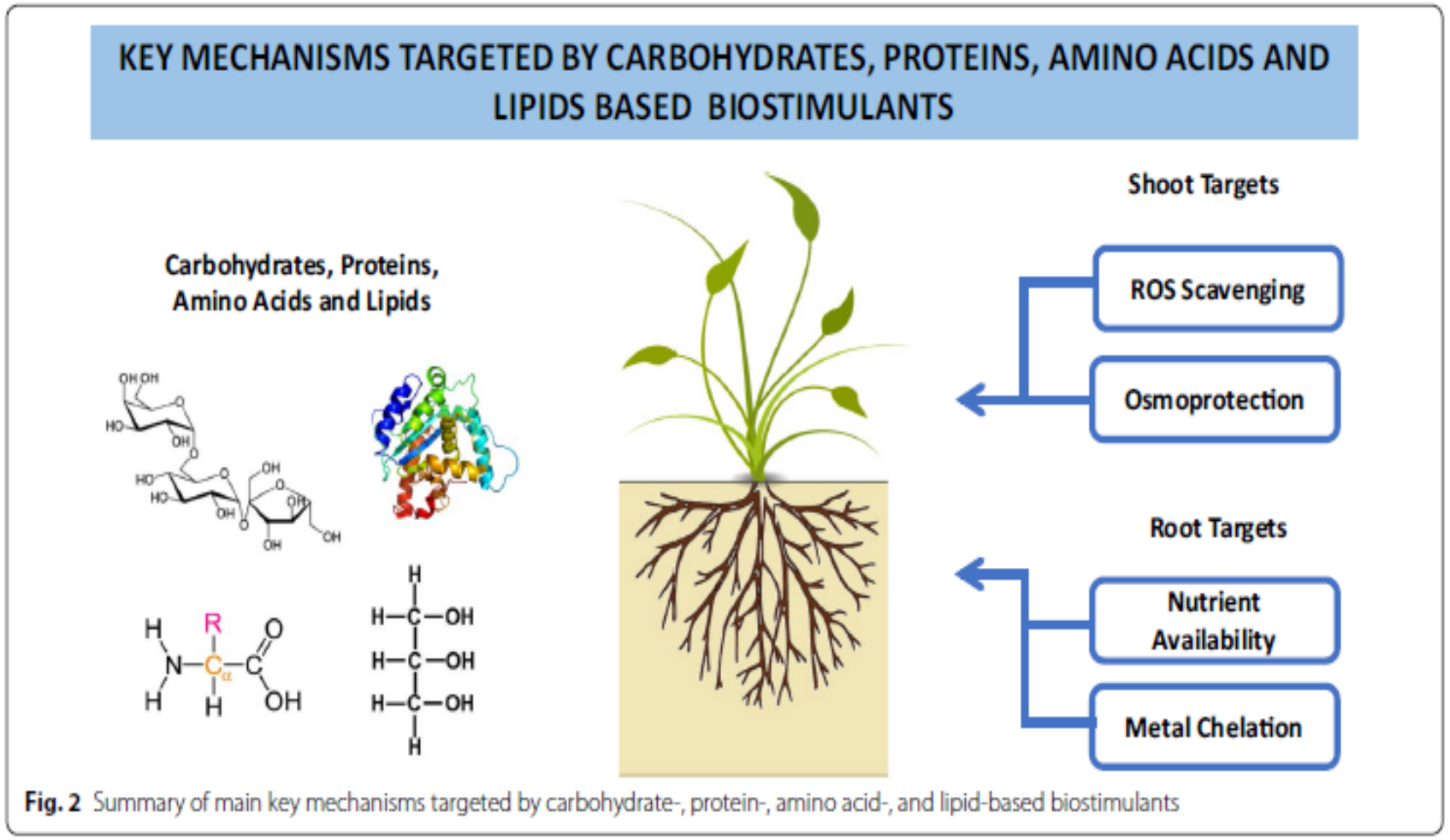

Fig. 2 Summary of main key mechanisms targeted by carbohydrate-, protein-, amino acid-, and lipid-based biostimulants

Humic and fulvic substances are the major organic components of lignites, soil, and peat. Humic and fulvic acids are produced by the biodegradation of organic matter resulting in a mixture of acids containing phenolate and carboxyl groups. Fulvic acids are humic acids with a higher oxygen content and lower molecular weight. A number of examples exist indicating the potential for these substances to improve abiotic stress tolerance in plants (Fig. 3).

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fulvic acid-induced disease resistance to *Botrytis cinerea* in table grapes may be mediated by regulating phenylpropanoid metabolism

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ARTICLE INFO

Keywords:
Treatment
Disease resistance
Phenolic compounds
Enzyme activity
Gene expression

ABSTRACT

Gray mold caused by *Botrytis cinerea* is a major postharvest disease of table grapes that leads to enormous economic losses during storage and transportation. The objective of this study was to evaluate the effectiveness of fulvic acid on controlling gray mold of table grapes and explore its mechanism of action. The results showed that fulvic acid application significantly reduced downy blight severity in table grapes without exhibiting any antifungal activity *in vitro*. Fulvic acid induced phenylpropanoid metabolism, as evidenced by accumulation of phenolic compounds and flavonoids, higher activities of phenylalanine ammonia-lyase (PAL), cinnamate-4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL), up-regulation of genes related to phenylpropanoid biosynthesis (*PAL*, *C4H*, *4CL*, *STS*, *ROMT* and *CHS*). Our results suggested that fulvic acid induces resistance to *B. cinerea* mainly through the activation of phenylpropanoid pathway and can be used as a new activator of plant defense responses to control postharvest gray mold in table grapes.

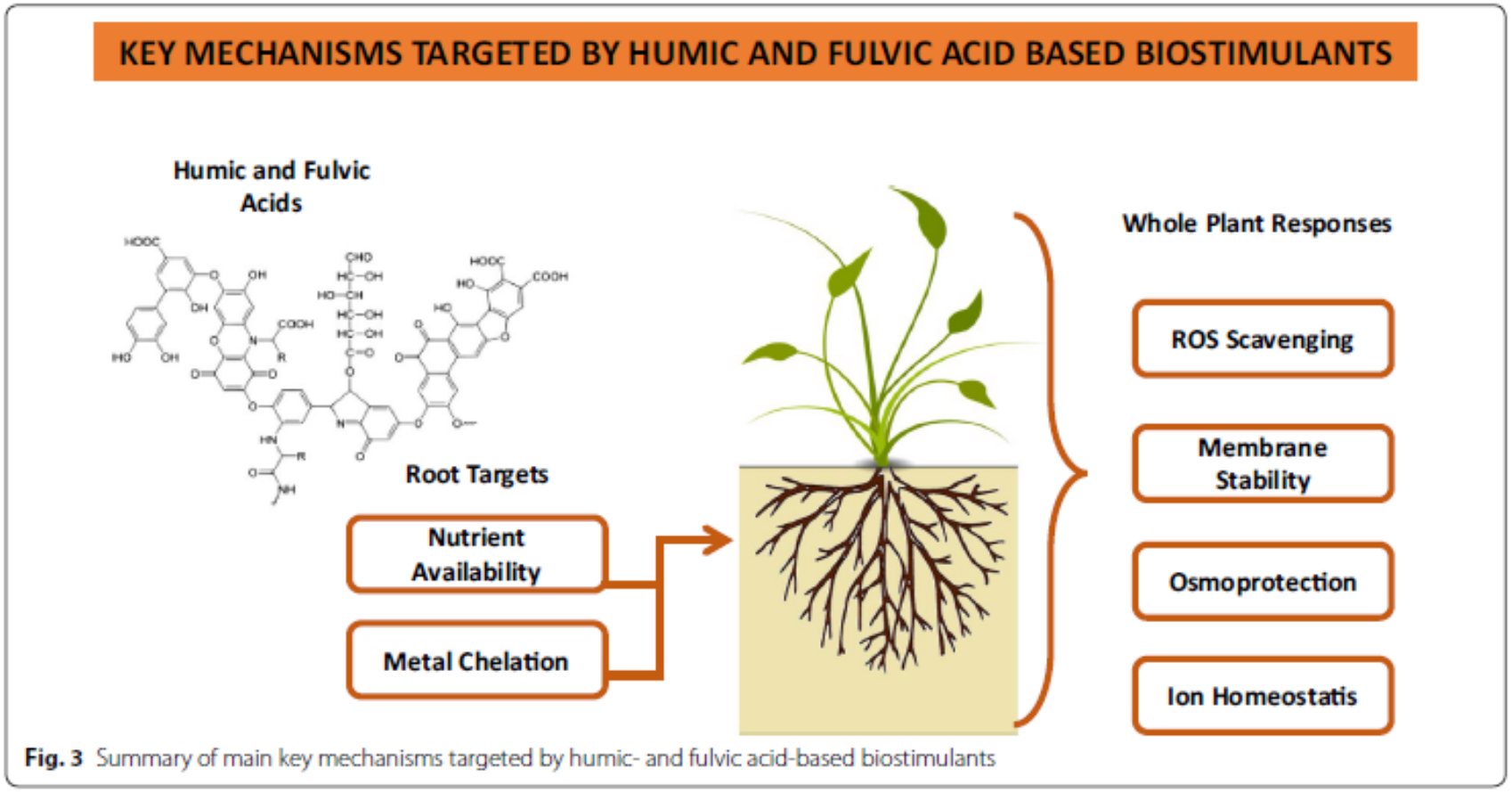

Fig. 3 Summary of main key mechanisms targeted by humic- and fulvic acid-based biostimulants

Microorganisms

While plants are known to establish symbiotic relationships with bacteria, our understanding of those relationships under abiotic stress is rudimentary. However, some of the targets of microorganisms that increase abiotic stress tolerance have been identified (Fig. 4). Bacteria with the potential to act as biostimulants have been isolated from a number of ecosystems with saline, alkaline, acidic, and arid soils. These bacteria belong to several genera such as *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas*, and *Bacillus*. Members of these genera have developed strategies to adapt and thrive under adverse conditions.

Food Research International
Volume 111, September 2018, Pages 416-422

Rhizobium and *Phyllobacterium* bacterial inoculants increase bioactive compounds and quality of strawberries cultivated in field conditions

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compounds, such as vitamin C or organic acids. Here we have analysed the effect on bioactive compounds in strawberries from plants biofertilized with the strains PEPV15 and PEPV16 in field conditions. Under these conditions, the anthocyanin content was increased when plants were biofertilized with the strain PEPV15 and the pelargonidin-3-*O*-rutinoside content significantly increased. Besides, citric acid, vitamin C and epicatechin contents were significantly higher when either of the two strains was used as biofertilizer. Our results showed that the inoculation with *Phyllobacterium* and *Rhizobium* strains is a good agronomical practice, which improve the content of several bioactive compounds of strawberries increasing the beneficial effects on human health.

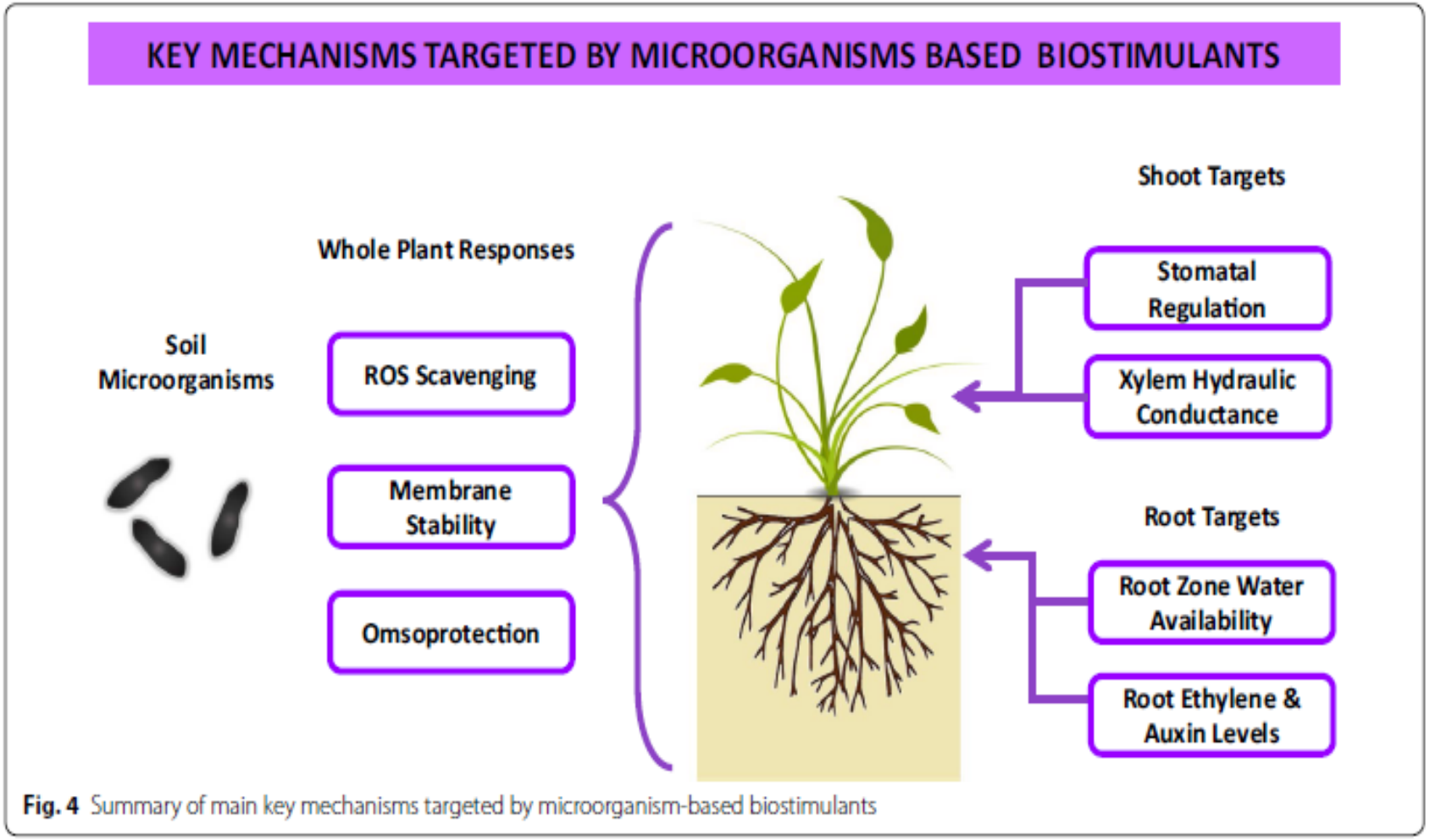

Fig. 4 Summary of main key mechanisms targeted by microorganism-based biostimulants

New biostimulants

Food processing by-products and wastes are abundant, cheap and rich sources of bioactive compounds of great interest to agriculture.[14] Besides their common use as compost and animal feed, a growing interest has been given recently to their valorization as biostimulants either as hydrolysates or extracts.[15,17] For such purposes, green extraction protocols have been developed allowing for a sustainable separation and extraction of compounds. Such techniques aim to reduce or eliminate the use of hazardous and polluting organic solvents and favour water as solvent. Although concentrations of compounds extracted may be lower than with other techniques, aqueous extracts have been demonstrated to be effective in the recovery of bioactive compounds.[18] In this view, water extracts of borage plants were able to enhance leaf pigment, yield, flavonoids and phenol content of lettuce.[19] In fact, water-dissolved compost organic matters also showed bioactive potential by increasing root and shoot growth, and enzyme activity of nitrogen metabolism of maize, similarly to the positive effects of aqueous soil organic matter extracts on spruce seedling growth.[20,21]

Environmental Regulation of Human Health Nutrients

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Three of the most important human health phytonutrients are vitamins (A = β-carotene, B9 = folic acid, and C = ascorbic acid) that are abundant and easily derived from fruits and vegetables (Craig and Beck, 1999).

HortScience Vol. 41(1) February 2006

The qualitative make-up of all fruits and vegetables is highly regulated by genetics

Once a cultivar is growing, the environment imparts a major influence on the concentration of all phytonutrients

Temperature

Ascorbic acid declines with increasing temperature, while lycopene, the principal carotenoid (coloring agent) in tomatoes, increases with an increase from low to medium temperatures, then drastically declines as temperatures increase from medium to high.

The overall general effects of temperature on ascorbic acid and β-carotene content in fruits and vegetables from these examples and others (Mozafar, 1994) can be summarized as follows: 1) most leafy vegetables and temperate fruits, grown at low versus high (30 °C) temperatures will have higher ascorbic acid concentrations; 2) an increase or decrease in air temperature of 4 °C, 4 to 5 d before harvest, has the greatest impact on postharvest ascorbic acid concentrations; 3) low temperatures increase ascorbic acid concentrations in produce, because degradation is reduced; and 4) temperature regulation of carotenoids is crop specific. In cool season crops such as carrot, maximum β-carotene synthesis occurs at 15 to 21 °C. In warm season crops such as guava (*Psidium guajava* L.), papaya, mango, and melon, optimum β-carotene synthesis occurs at 30 °C. Carotenoid synthesis in tomato is even more temperature dependent; maximum lycopene synthesis occurs at 25 to 30 °C and is inhibited above 32 °C (Tomes, 1963). In watermelon (*Citrullus lanatus* (Thunb.) Matsumura and Nakai), lycopene synthesis is inhibited above 37 °C

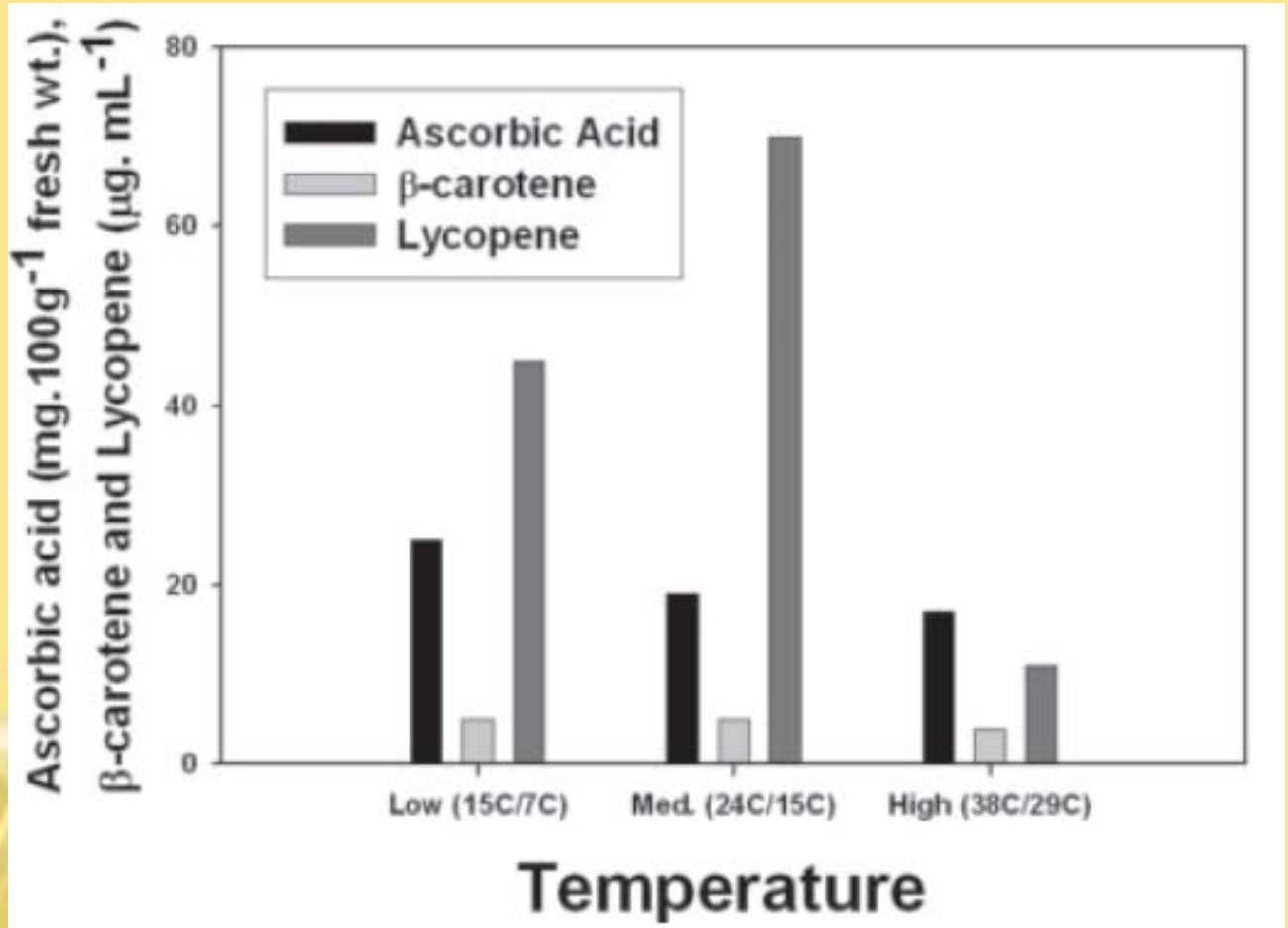


Fig. 1. Ascorbic acid, β-carotene, and lycopene content of vine ripened tomato 'John Baer' fruit grown at low vs. high day/night temperatures (Sayre et al., 1953).

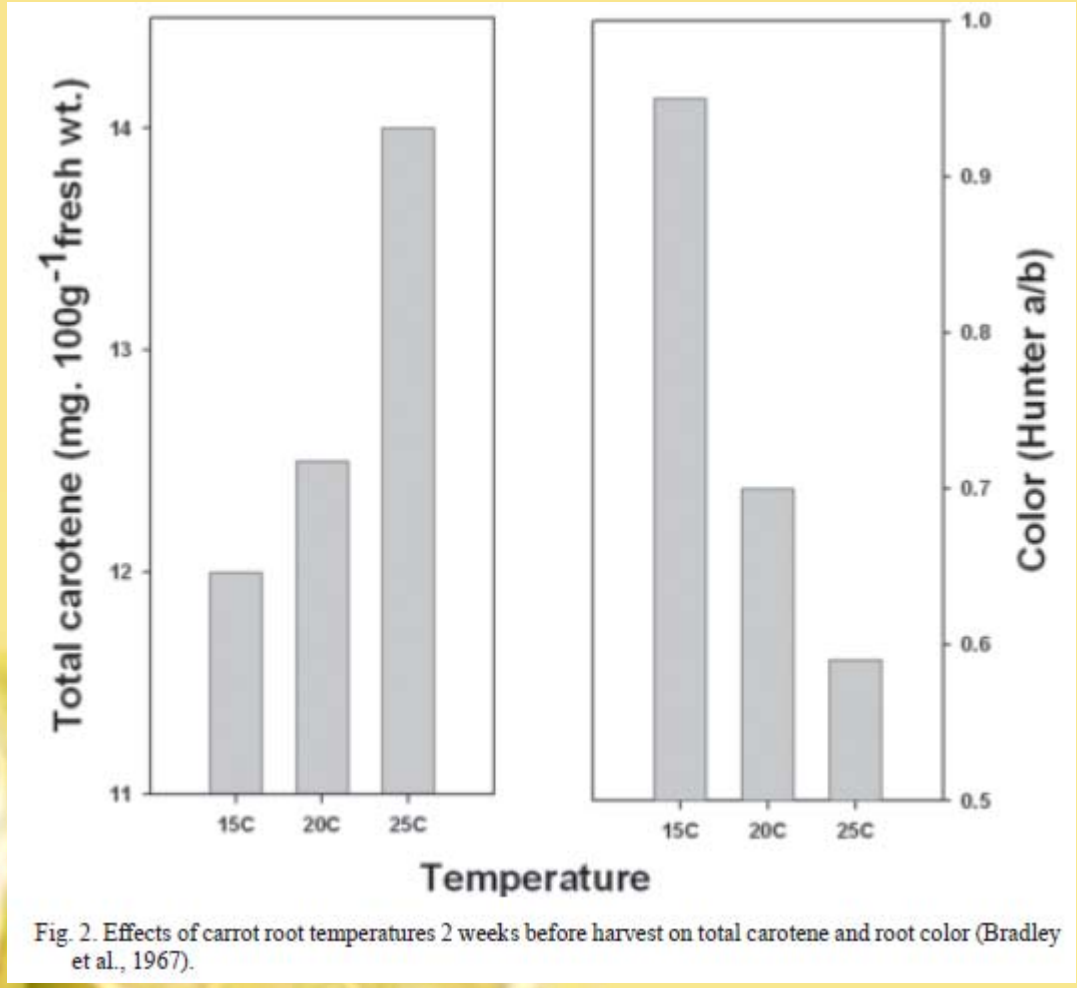


Fig. 2. Effects of carrot root temperatures 2 weeks before harvest on total carotene and root color (Bradley et al., 1967).

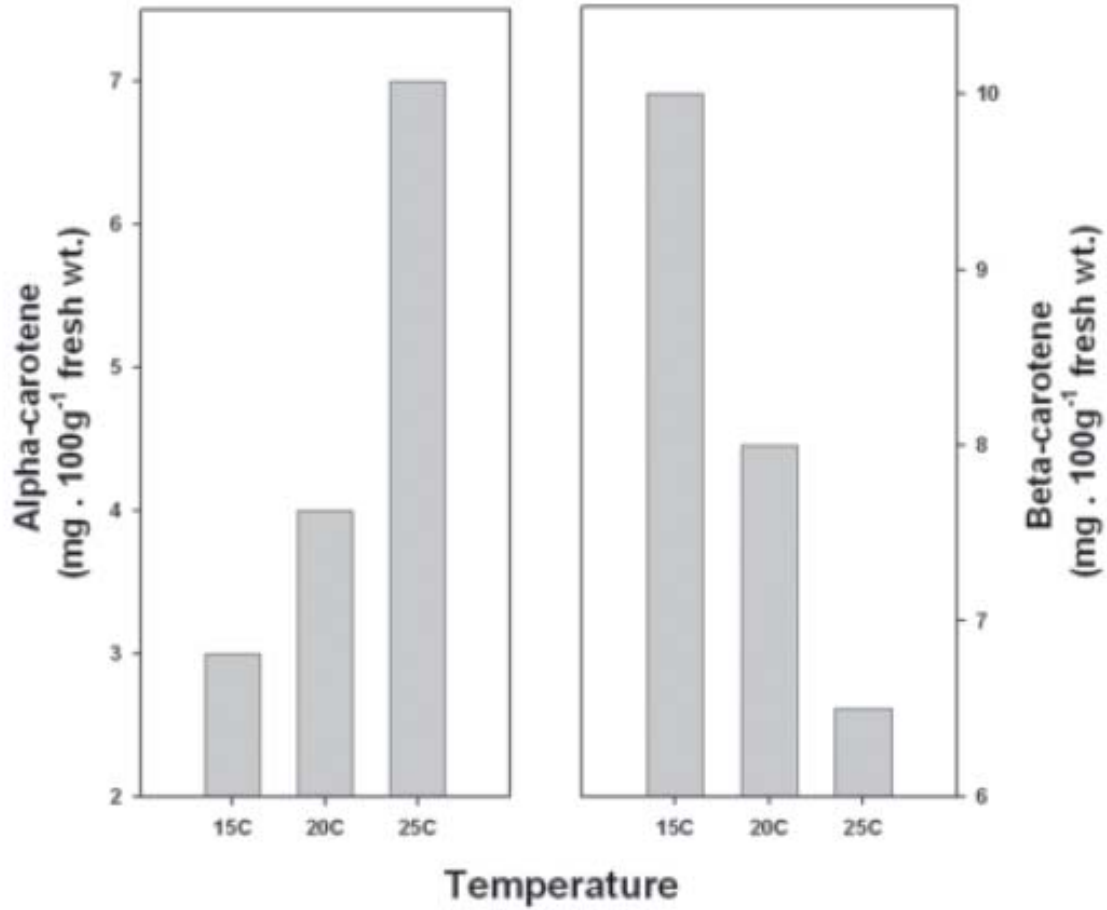


Fig. 3. Effects of carrot root temperatures 2 weeks before harvest on α-carotene and β-carotene (Bradley et al., 1967).

Light. Light intensity and light quality are extremely influential in regulating fruit and vegetable vitamin content. Mustard greens grown under full sunlight versus 50% sunlight were significantly lower in ascorbic acid concentration, and significantly higher in total carotene and total chlorophyll concentrations (Fig. 4) (Makus and Lester, 2002). Findings from this mustard-leaf light study demonstrated that 1) reduced light intensity reduces the synthesis of glucose, the starting molecule in ascorbic acid biosynthesis, and the ascorbic acid concentration declined; 2) reducing light intensity reduces leaf temperature, which is favorable for β-carotene synthesis; 3) increased β-carotene content increases the protection of chlorophyll from photo-bleaching, thus increasing chlorophyll concentration; and 4) light intensity appears to have little influence on folic acid content in mustard greens. Several studies on a variety of fruits and vegetables such as apple (Zhi-Qiang et al., 1999), broccoli (Krumbien and Schonhof, 1999), citrus (Izumi et al., 1992), green bean leaves (Schmitz-Eiberger and Noga, 2001), grape (Uhlig-Birgit, 1998), pepper (Simkin et al., 2003), and spinach (Nakamoto et al., 1998) have examined the effect of light quality and found that 1) supplementing natural light with blue or sodium vapor light increases ascorbic acid concentration; 2) UV-B radiation decreases both ascorbic acid and β-carotene concentrations; and 3) red light greatly stimulates lycopene synthesis in tomato, while far-red light inhibits lycopene synthesis (Alba et al., 2000; Cookson et al., 2003).

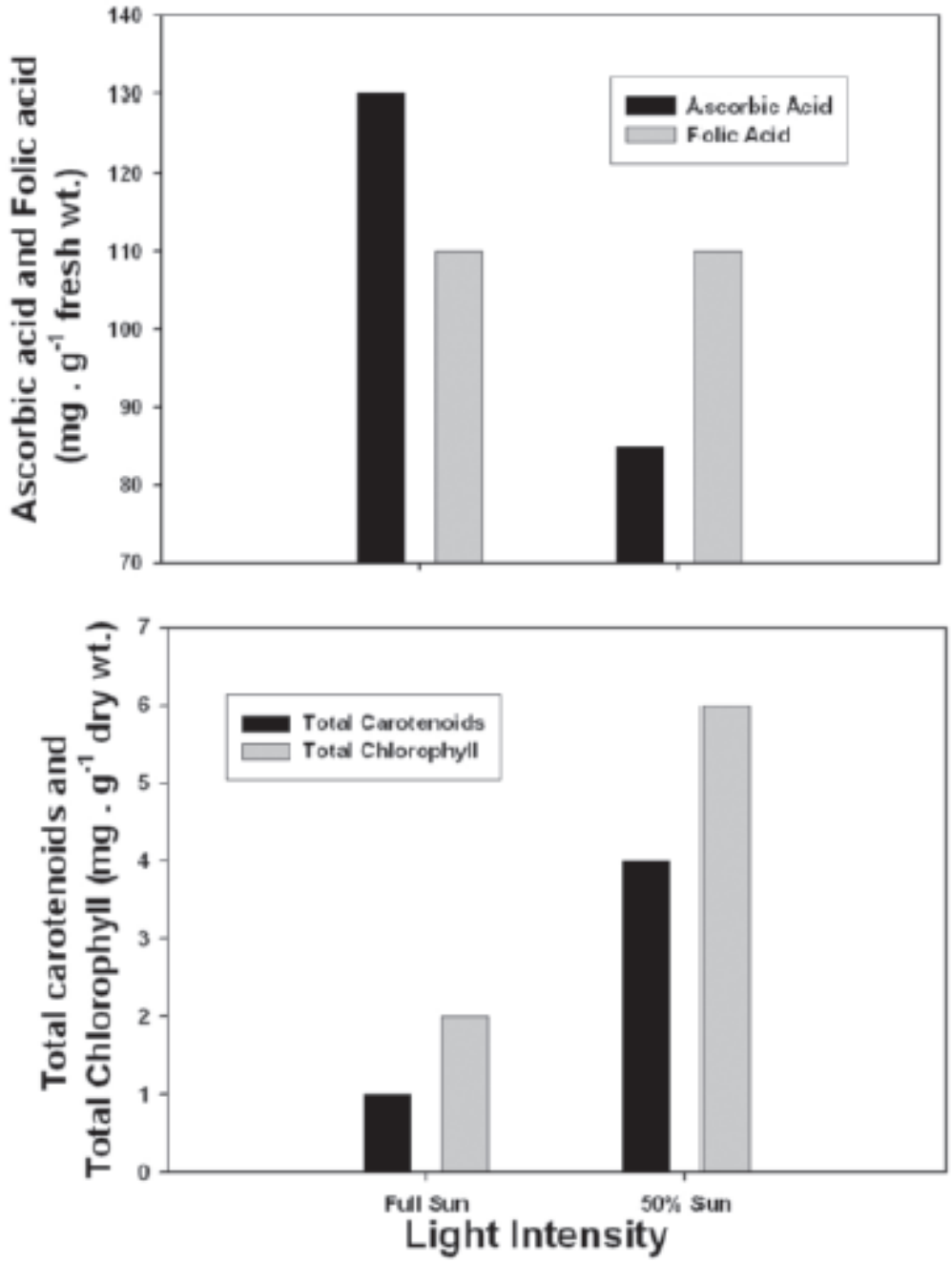
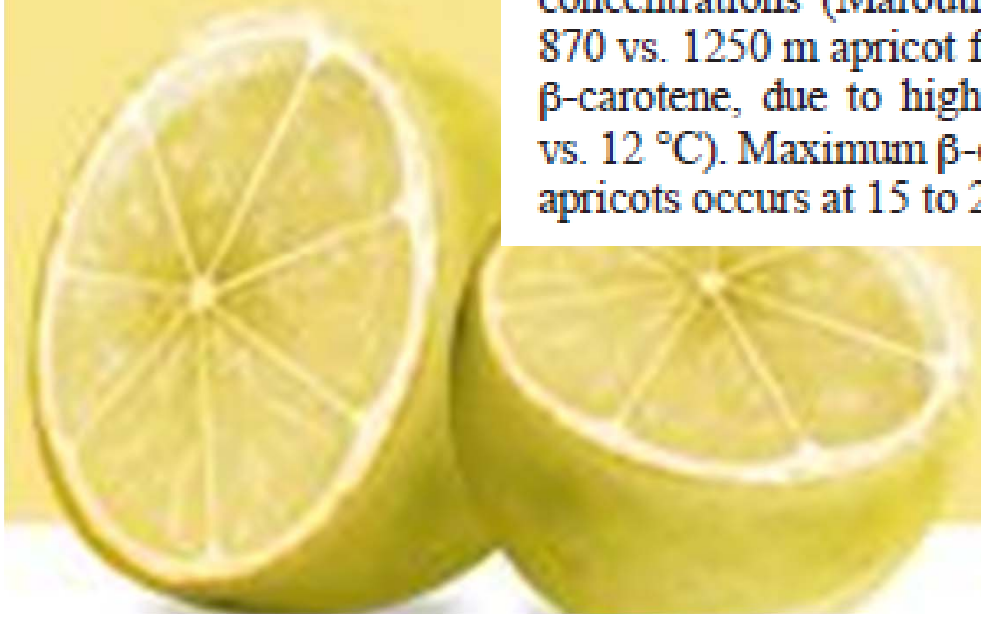


Fig. 4. Effects of growth under full sunlight or 50% shade on ascorbic acid, folic acid, β-carotene, and total chlorophyll of mustard greens (Makus and Lester, 2002).

Altitude. Effects of altitude are due to a combination of light and temperature. Altitude is particularly important as a cultural consideration in hilly or mountainous agricultural regions. Apples grown at higher altitudes contain higher ascorbic acid levels (Feteliyer, 1977). At 1000 vs. 50 m, apples contained 2-fold more ascorbic acid due to higher light intensity (140 vs.160 mW), and also likely due to lower temperatures which were not reported. However, apricots grown at higher altitudes contain lower β-carotene concentrations (Maroutian et al., 1985). At 870 vs. 1250 m apricot fruit had 2-fold more β-carotene, due to higher temperatures (20 vs. 12 °C). Maximum β-carotene synthesis in apricots occurs at 15 to 21 °C.



Scientia Horticulturae 216 (2017) 12–21

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Review

An overview of preharvest factors affecting vitamin C content of citrus fruit

Lembe Samukelo Magwaza[a,*], Asanda Mditshwa[b], Samson Zeray Tesfay[b], Umezuruike Linus Opara[c,d]

2. Preharvest factors affecting AsA content

There is enough literature suggesting that appropriate postharvest management practices of citrus fruit can decrease postharvest nutritional losses and enhance fruit quality. However, several studies have indicated that fruit biochemical composition and quality are mostly affected by preharvest factors, such as scion-rootstock combination, carbohydrate content (Goldschmidt, 1999), crop load and fruit position within the tree canopy (Volz et al., 1993), light and temperature (Ferguson et al., 1999). Genetic make-up, cultural practices, and environmental conditions that result in an increase in fruit ROS will also influence AsA content at harvest (Davey et al.,

2.1. Cultivar

The chemical and nutritional attributes of citrus fruit, including carotenoids and AsA content, are mainly determined by multigenic inheritance (Fanciullino et al., 2006). Different studies have shown that the levels of AsA in citrus fruit juice sacs are highly variable, ranging from 16.2 to 46.2 mg/mL juice depending on species and cultivar (Table 1) (Sharma et al., 2006; Lafuente et al., 2011; Sdiri et al., 2012).

Mandarins (*Citrus unshiu* Marc.) and oranges (*Citrus sinensis* Osb.) are the two commercial species which have been shown to have a significant difference in AsA levels (Nagy, 1980). The AsA composition and content in the pulp of oranges is generally higher than in mandarins. The lower contents of AsA in mandarins compared to oranges were observed by Dhuique-Mayer et al. (2005) and recently confirmed in an analysis by Yang et al. (2011) which showed that the average AsA contents in fruit juice sacs of mandarin and orange at the maturity stage were 27.3 and 48.9 mg/100 mL juice, respectively. Variances in AsA concentration between mandarins and oranges have been suggested to be linked to the differentials in the expression of genes encoding for enzymes involved in the L-galactose pathway during ripening and the difference in the activity of AsA oxidation and recycling enzymes (Yang et al., 2011 Alós et al., 2014). In general, Martí et al. (2009) and Escobedo-Avellaneda et al. (2014) showed that oranges, generally have the highest AsA levels (29 and 82 mg/100 mL of juice), followed by lemons (30–50 mg/100 mL), grapefruits (30–60 mg/100 mL) and mandarins (20–60 mg/100 mL).

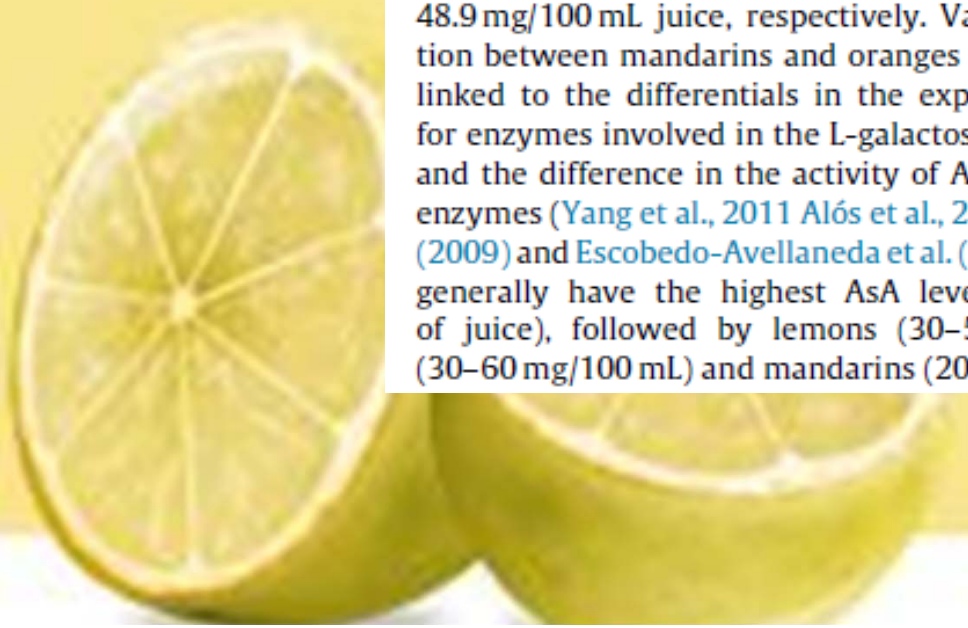

Table 1
Vitamin C concentration of different citrus types and cultivars.

Citrus species	Cultivar	Vitamin C (mg/100 mL)	Reference
Mandarin	Kinnow	18.2	Sharma et al. (2006)
	Nagpur	16.2	
	Cleopatra	20.2	
	Kaula	19.5	
	Safor	29.0	Sdiri et al. (2012)
	Garbí	21.2	
	Fortune	29.8	
	Kara	25.5	
	Murcott	23.5	
	Fortune	28.3 – 31.6	Lafuente et al. (2011)
Sweet oranges	Mosambi	22.2	Sharma et al. (2006)
	Pineapple	22.8	
	Blood Red	20.8	
	Valencia Late	18.9	
	Jaffa	21.5	
Lemon	Eureka	39.6	Sharma et al. (2006)
	Lisbon	42.3	
	Kagzi Kalan	40.8	
Lime	Kagzi	46.2	Sharma et al. (2006)
Grapefruit	Foster	23.6	Sharma et al. (2006)
	Duncan	21.2	
	Marsh	22.8	
Pummelo	Local	19.2	Sharma et al. (2006)
	Kaoopan	21.6	
	China	21.0	
Tangelo	Thornton	19.2	Sharma et al. (2006)

Table 2
Vitamin C content in different edible tissues of citrus fruit of four species.

Citrus species	Edible tissues	Vitamin C (mg/100 g FW)
C. unshiu	Juice sack	25.4
	Segment membrane	32.6
	Segment	26.1
C. reticulata	Juice sack	45.3
	Segment membrane	14.2
	Segment	38.9
C. sinensis	Juice sack	32.9
	Segment membrane	35.2
	Segment	35.2
C. changshanensis	Juice sack	41.6
	Segment membrane	15.7
	Segment	32.7

Table 3
Ascorbate contents in peel and pulp different citrus types and cultivars.

	Pulp	Peel	
	mg mL^{-1}		
Lima orange	46.1	43.2	Barros et al. (2012)
Pera orange	68.1	24.3	
Tahiti lime	41.4	6.84	
Sweet lime	60.2	22.6	
Ponkan mandarin	41.1	47.6	
	μmol g^{-1} FW		
Egan No.2 orange	11.33	2.03	Yang et al. (2011)
Guoqing No.1 orange	8.76	1.56	
Newhall mandarin	10.52	3.69	
Dream mandarin	8.76	3.44	

2.2. Rootstocks

2.3. Rainfall and irrigation

The accumulation of vitamin C has been shown to be negatively correlated with irrigation and rainfall (Toivonen et al., 1994). In a study by Navarro et al. (2015), the concentration of AsA in the juice of 'Star Ruby' grapefruits was not affected by water stress treatments. A similar trend was also observed by Buendía et al. (2008).

However, some studies have reported that reducing water availability to 50% of the crop requirement significantly increased the AsA content in mandarin (Navarro et al., 2010; Panigrahi et al., 2014) as well as tomato fruits (Favati et al., 2009). Navarro et al. (2010) showed that if deficit irrigation treatment was imposed during phase II of fruit growth, the concentration of AsA was 15% higher than the control. From these observations, the increase in concentration could either result from biosynthesis of AsA, in response to water stress, or the lower fruit water content, resulting from the lower plant water potential (Navarro et al., 2010). Water deficit during the stage II and III of fruit growth increase soluble sugar accumulation in 'Satsuma' mandarin fruit (Yakushiji et al., 1996).

2.4. Light and temperature

Direct exposure of fruit on the outside position of the canopy to sunlight may have led to an up-regulation of the AsA biosynthetic and recycling pathways (Ma and Cheng, 2004). Considering that carbohydrates produced during photosynthesis are precursors of AsA synthesis, it can also be hypothesized that lower AsA in the peels of sun-shaded fruit may have resulted from reduced photosynthesis due lower photosynthetically active radiation on shaded portions of the tree canopy. As a potent antioxidant in citrus fruit (Mathur et al., 2011; Sdiri et al., 2012), a higher concentration of AsA observed in the peel of fruit harvested from the sun-exposed position of the canopy may also suggest a defense mechanism against preharvest stresses.

According to the discussion in the Introduction section (Section 1) of this review, AsA is synthesized from carbohydrates precursors (Fig. 1). Therefore, a lower concentration of sugars in shaded fruit inside the canopy could contribute to lower levels of AsA compared to sun-exposed outside fruit (Valpuesta and Botella, 2004; Zhan et al., 2013). A positive correlation between AsA and sucrose and glucose, by Magwaza et al. (2013) confirmed the role of these sugars, especially glucose as a primary substrate for AsA synthesis pathway (Valpuesta and Botella, 2004).

2.5. Fertilization

2.5.1. Macronutrients

The effect of tree nutrition on internal fruit quality and storage ability of citrus fruits are significant and well known. High nitrogen (N) fertilization of citrus fruit trees has been reported to be associated with significant reduction in levels of AsA (Lee and Kader, 2000). Increasing N and phosphorus (P) supply to citrus trees have been shown to results in lower AsA content in fruit while

Reduced vitamin C content in plants receiving high N rates may be due to increased vegetative growth and large fruit, generally improved by the N fertilization leading to the occurrence of a dilution effect in the plant tissues (Lee and Kader, 2000). Furthermore, reduction in AsA as a result of N fertilization may results from reduced light intensity and accumulation of AA in shaded parts due to increased foliage (Lee and Kader, 2000). However, Chen et al. (1999) reported an increase in Ascorbic acid content in fruit

Phosphorus is well known to influence fruit internal quality (Nagy, 1980). Bar-Akiva et al. (1967) reported a positive correlation between phosphorus and vitamin C content of grapefruit. Contrasting results were reported by Dou et al. (2005) who showed that in grapefruit, vitamin C was the highest when trees received with optimum K (186 kg/ha) without P. Optimal P (48 kg/ha) application without K significantly reduced juice vitamin C concentration. Similar results were reported by Mann and Sandhu (1988) who found that in 'Kinnow' mandarins, vitamin C was increased by K, but not by P application.

Potassium is regarded as an essential element required for a consistent production of high quality citrus fruits. Nagy (1980) reported that K effects on citrus fruit quality are more important than its effect on yield. Several studies have shown that high levels of K fertilization increase vitamin C in lemons (Embleton and Jones, 1966), oranges (Reitz and Koo, 1960), and grapefruit (Smith

2.5.2. Micronutrients

The concentration of AsA is not uniquely affected by macronutrients. Several studies have reported the effect of micronutrients on the improvement of ascorbic acid of citrus fruit. Mishra et al. (2003) evaluated the effect of zinc (Zn) and boron (B) on yield and quality 'Kinnow' mandarins. Fruit harvested from trees receiving Zn (0.5%) + B (0.4%) micronutrient treatments had higher AsA. Similar results were observed by Babu et al. (1984) who reported higher AsA concentration in 'Kagzi' limes (*C. aurantifolia* Swingle) when Zn (0.6 percent) and 2,4-D (20 ppm) were applied during spring and summer flushes.

2.6. Hormones and plant growth regulators

Application and influence of plant growth regulators (PGRs) such as gibberellic acid (GA_3) and cytokinins for production of quality fruit is well documented for mandarins (Garcia-Luis et al., 1985; Pozo et al., 2000; Khalid et al., 2012), grapefruit (El-Zeftawi, 1980), and oranges (Fidelibus et al., 2002). The beneficial effects of hormones such as GA_3 on fruit quality was reported by investigators such as Morton (1981) who found that GA_3 either applied at full flowering or at small fruit stage, significantly increased the number of harvested 'Washington' navel fruits.

Similarly, Nodiya and Mikaberidze (1991) reported that Satsuma mandarin fruits treated with 60 ppm of gibberellic acid before the fruit showed signs of yellowing had a higher accumulation of vitamin C content. This is consistent with the statement by Eassa et al. (2012) who reported that improved fruit vitamin C content may be due to its role in enhancement the nutritional status reflected on yield and fruit quality. Kuiper (1993) suggested that this is mostly because the sink strength is established and regulated by plant growth regulators. Plant hormones such as cytokinins and gibberellic acid have been found to increase movement of photosynthates to fruit and regulate several rate-limiting constituents involved in carbon partitioning (Ozga and Dennis, 2003). Brenner and Cheikh (1995) argued that PGRs may either stimulate mobilization of nutrients through the phloem, adjust the sink strength of the tissue by enhancing its growth and increase the capacity for unloading of sugars from the phloem or they may act on metabolism and compartmentalization of sugar and its secondary metabolites.

Some scientists reported the inconsistent effect of PGRs on ascorbic acid contents of citrus fruit (Lima and Davies, 1984). Using young (3–5 years) trees, Khalid et al. (2012) investigated the effect of PGRs, including benzyladenine (BA), kinetin and GA_3 applied at different growth stages on the quality of 'Kinnow' mandarins. These authors reported that the PGRs had a significant negative influence on juice ascorbic acid with maximum AA contents (58.45 mg 100 mL^{-1}) of juice observed on the control treatment while minimum AA contents (34.88 mg 100 mL^{-1}) were observed

Scientia Horticulturae 218 (2017) 95–104

Contents lists available at ScienceDirect

Scientia Horticulturae

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Postharvest factors affecting vitamin C content of citrus fruits: A review

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Table 1
Effects of ethylene degreening treatment on vitamin C content of citrus fruits.

Ethylene treatment	Produce	Effect ethylene degreening on vitamin C	References
2 ppm (60 h)	'Star Ruby' grapefruit	Degreened fruit had lower vitamin C content	Chaudhary et al. (2012)
3.5 ppm (72 h)	'Rio Red' grapefruit	Vitamin C was higher in degreened fruit	Chaudhary et al. (2015)
4 ppm (72 h)	'Navel' Orange, 'Star Ruby' grapefruit and 'Miho Satsuma' mandarins	Ethylene degreening had no effect on vitamin C content	Mayuoni et al. (2011)
2000 ppm (120 h)	'Navelina' orange, 'Clemenules' Clementines, 'Clemenpons', 'Oronules' and 'Basol'	The treatment has no effect vitamin C	Sdiri et al. (2012)
10 ppm (48 h)	'Mosambi' orange	Vitamin C content of the degreened fruit was not different from non-degreened fruit	Ladaniya and Singh (2001)
2 ppm (5 min)	'Sinnari' orange	Degreening treatment reduced vitamin C content	Elkashif et al. (2015)

Table 2
Effects of surface coatings and waxing on vitamin C content of citrus fruits.

Treatment	Produce	Effect of treatment on vitamin C	References
Arabic gum and olive oil	Sweet lemon	Arabic gum coating had higher vitamin C retention compared to untreated fruit. Olive oil reduced vitamin C content	Eskandari et al. (2014)
Carnauba wax	'Delta Valencia' orange	Insignificant difference between waxed fruit and control treatment	Pereira et al. (2013)
Carnauba wax-GFSE	'Satsuma' mandarin	Compared to wax without GFSE, GFSE-enriched wax had better vitamin C retention	Shin et al. (1998)
Chitosan	Tankan	Resulted in higher vitamin C retention	Chien and Chou (2006)
Chitosan (Low molecular weight; Mw = 15 kDa)	'Murcott' Tangor	Low molecular weight chitosan (Mw = 15 kDa) increased vitamin C of tangor fruit stored at 15 °C for 56 days	Chien et al. (2007)
Coconut oil	'Kagzi' Lime	Coconut oil reduced vitamin C loss during storage	Bisen and Pandey (2008), B et al. (2012)
Emulsion with paraffin wax, emulgin PE, water, and carboxymethyl cellulose	Mandarin	The coating delayed the loss of ascorbic acid during the 28 days at 25 °C and 75% RH	Toğrul and Arslan (2004)
Hydroxypropyl methylcellulose-beeswax-shellac coating	'Valencia' orange	The coating had no effect on vitamin C levels	Contreras-Oliva et al. (2011
Irradiated chitosan	'Kinnow' mandarin	Chitosan significantly minimized the loss of ascorbic acid following 3 months of storage at 4 °C and 80% RH	Abbas et al. (2008)
Nipro Fresh SS 40T and SS 50′	'Kinnow' mandarin	Fruit coated with 'Nipro Fresh SS 40T and SS 50′ had delayed loss of vitamin C	Mahajan et al. (2013)
Polysaccharide-based coating and shellac wax	'Valencia' orange	Both waxes did not affect vitamin C	Baldwin et al. (1995)
SemperfreshTM	'Miho' mandarin	Semperfresh coating did not significantly affect vitamin C	D'Aquino et al. (1996)
SemperfreshTM and JonfreshTM	'Satsuma' mandarin	Both coatings were effective in retaining vitamin C	Bayindirli et al. (1995)
Shellac wax	'Satsuma' mandarin	Shellac wax had no effect on vitamin C retention.	Shen et al. (2013a)
Wax	'Valencia' oranges	No significant effect on vitamin C retention	Ansari and Feridoon (2007)
Wax	'Siavarz' orange	The wax had higher vitamin C retention on fruit after 90 days of storage.	Ansari and Feridoon (2007)
Wax + Citral	'Ponkan' mandarin	Fruit treated with citral enriched wax had better vitamin C retention compared to those without citral.	Fan et al. (2014)

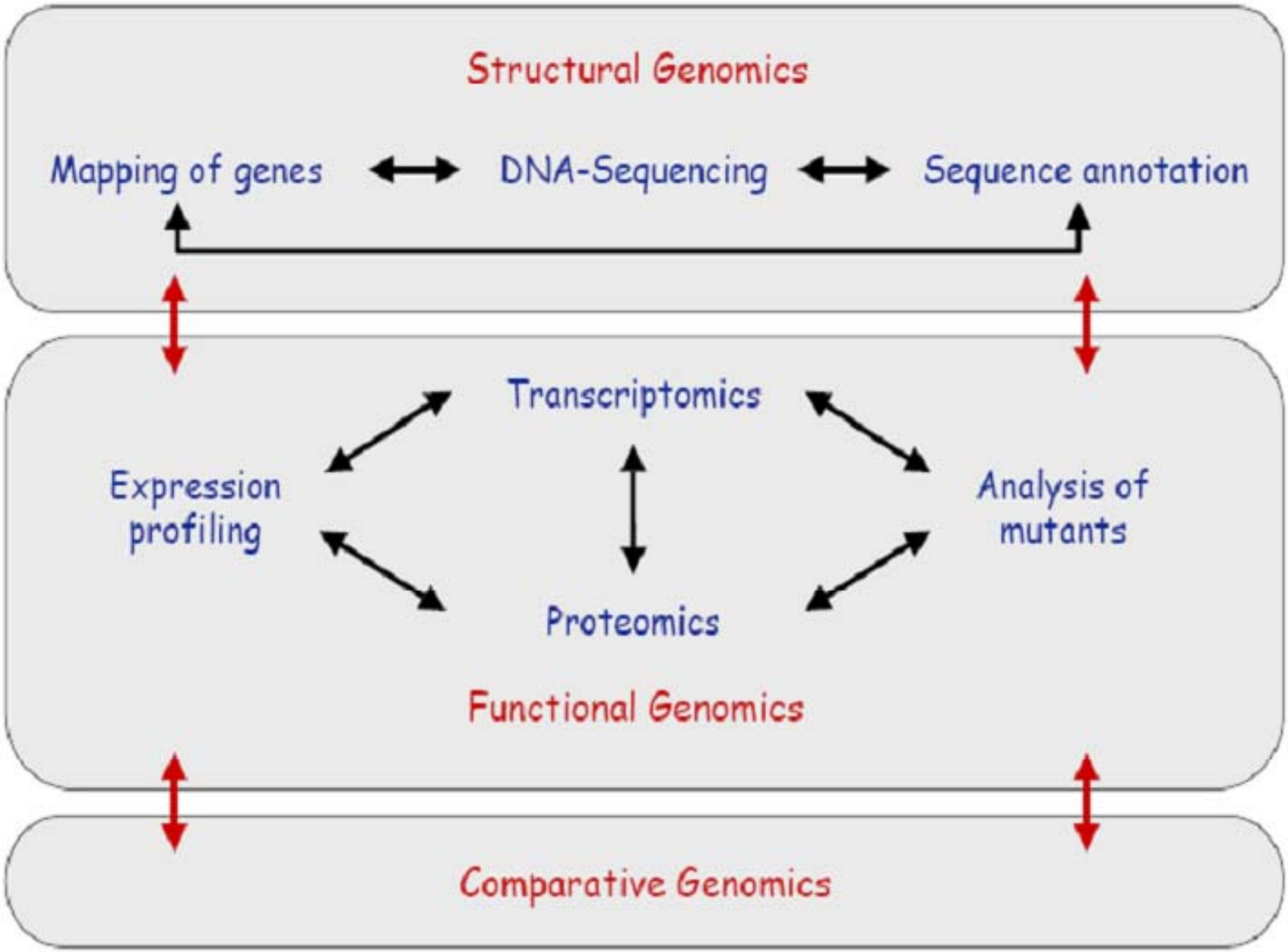
Table 3
Effects of postharvest irradiation treatments on vitamin C content of citrus fruits.

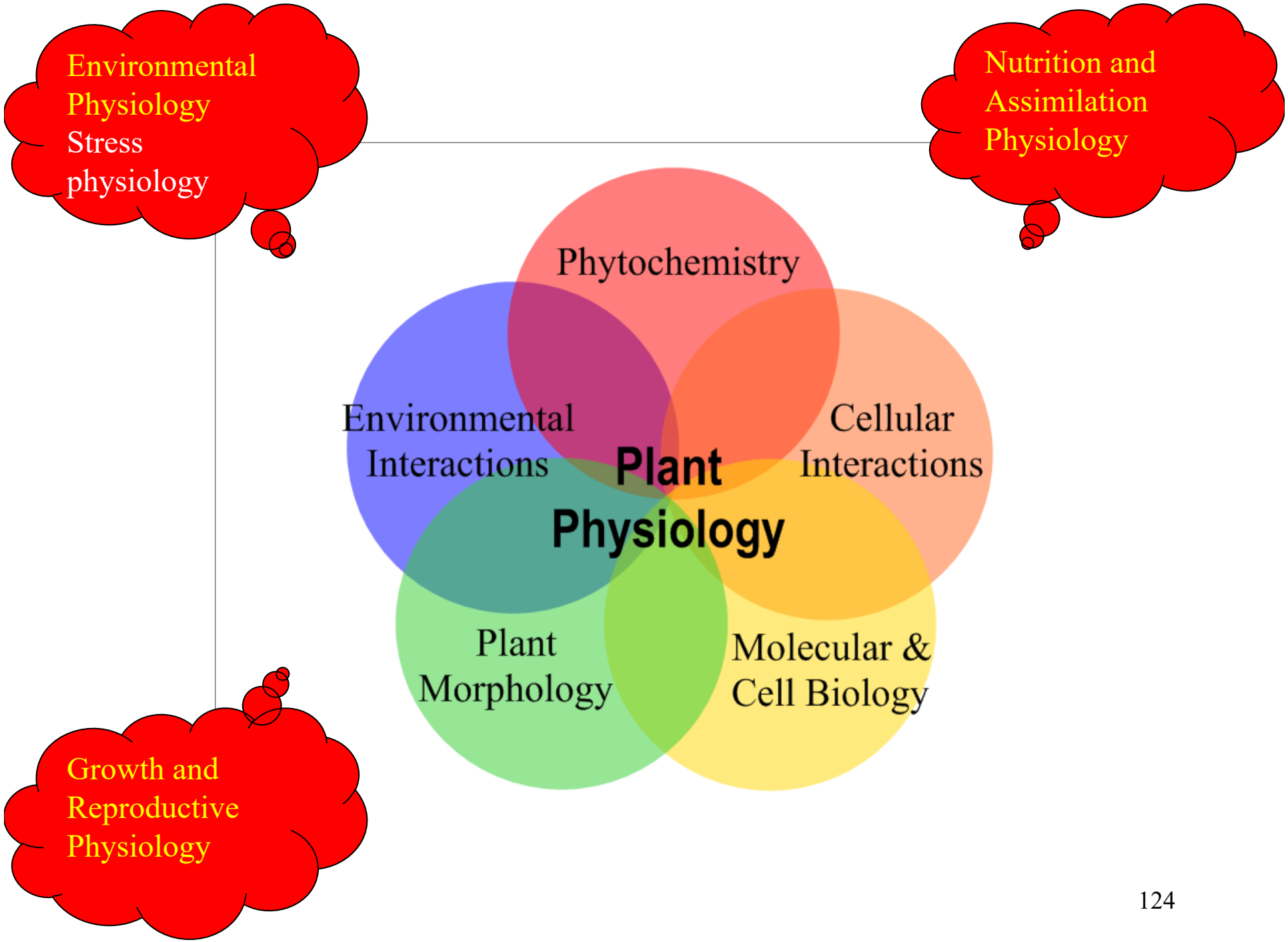
Irradiation Treatment	Produce	Effect of irradiation on vitamin C	References
Blue LED light (50 μmol $m^{-2}s^{-1}$)	'Satsuma' mandarins, 'Valencia' ranges, and 'Lisbon' lemon	Blue LED light treatment significantly increased the ascorbic acid content in the juice sacs	Zhang et al. (2015)
Electron-beam (1 kGy)	'Rio Red' and 'Marsh White' grapefruit	Irradiation had no effect on vitamin C content, however, doses more than 1 kGy led to marked reduction in vitamin C content	Girennavar et al. (2008)
Gamma rays (10–200 Gy)	'Valencia' oranges	Irradiated and non-irradiated fruit had similar vitamin C content	De Bortoli et al. (2015)
Gamma rays (0.25–1.5 kGy)	'Nagpur' mandarins, 'Mosambi' sweet orange, 'Kagzi' acid lime	All irradiated fruit had significantly lower vitamin C content than untreated fruit	Ladaniya et al. (2003)
Gamma rays (0.3 kGy)	'Moroccan' Clementine mandarins	Irradiation significantly increased vitamin C content	Mahrouz et al. (2002)
Gamma rays (0.5 kGy)	'Blood red' oranges	Vitamin C retention was high in irradiated fruit compared to control treatment or fruit irradiated at 0.25 kGy	Khalil et al. (2009)
Gamma rays (200–600 Gy)	'Lane Late' Navel oranges	All the irradiation doses had no effect on vitamin C retention	McDonald et al. (2013)
Gamma rays (300–700 Gy)	'Rio Red' grapefruit	Irradiation had no significant effect on vitamin C levels of early-season 'Rio Red' grapefruit	Patil et al. (2004), Vanamala et al. (2005)
Red LED light (100 μmol $m^{-2}s^{-1}$)	'Satsuma' mandarins, 'Valencia' oranges, and 'Lisbon' lemon	Red LED light treatment had no effect on ascorbic acid content of the fruit	Zhang et al. (2015)
UV-B (19.0 kJ m^{-2})	'Tahitian' lime	UV-B irradiation significantly delayed the loss of ascorbic acid during storage	Kaewsuksaeng et al. (2011)
UV-C (3.94 J cm^{-2})	'Duncan' grapefruit	UV-C treatments caused a 25–35% reduction in ascorbic acid	La Cava and Sgroppo (2015)
X-ray (30–164 Gy)	'Clemenules' Clementine mandarins	Irradiation treatment had no effect on vitamin C content	Contreras-Oliva et al. (2011a)
X-ray (0.2–1 kGy)	'Navel' oranges	Vitamin C was not significantly different between irradiated and non-irradiated fruit	Cho et al. (2015), Noh et al. (2016)

Table 4
Effects of MAP on evolution of vitamin C content of citrus fruits.

Produce	Type of film	Atmospheric composition		Effect on vitamin C content	References
		O2	CO2		
'Star Ruby' grapefruit	Microperforated and macroperforated Xtend®	Not reported		Both treatments had no effect on vitamin C content	Chaudhary et al. (2015)
'Kay' lime	HDPE	Not reported		Vitamin C was better retained in HDPE bags with 1 × 40 microperforation compared unpackaged fruit	Ramin and Khoshbakhat (2008)
'Avana' mandarins	Yang PT 65	13.3%	8.8%	Fruit segments stored in Yang PT 65 film had higher vitamin C retention compared to those stored in Cryovac MY 15	Piga et al. (2002)
'Kinnow' mandarins	Cryovac heat-shrinkable film	13.2%	4.1%	Heat shrinkable RD-106 film had higher vitamin C retention compared to LDPE or HDPE	Mahajan et al. (2016)
'Kinnow' mandarins	Cling film	Not reported		Wrapping fruit with cling film rather than shrink film resulted in lower vitamin C content	Mahajan and Singh (2014)
'Malvasio' mandarins	Goglio-LDPE	2 kPa	13 kPa	Mandarins wrapped with Goglio-LDPE had lower vitamin C content compared to Cryovac film or control treatment	D'Aquino et al. (2001)
'Champagne' orange	PVC film and gelatin-based edible film	Not reported		Packaging films did not influence vitamin C content of minimally processed oranges	Agostini et al. (2013)
'Malta' orange	Polyethylene	Not reported		Polyethylene bags with 0.0508 mm thickness better preserved vitamin C compared to those with 0.0254 mm or control treatment	Hussain et al. (2004)
'Sinnari' orange	Non-perforated polyethylene film	Not reported		Compared to perforated polyethylene film and waxing, fruit wrapped with intact polyethylene film had higher vitamin C retention	Elkashif et al. (2015)
'Miho' Satsuma	Cryovac MD 15 film	Not reported		Wrapping fruit with cryovac film did not influence vitamin C content during storage	D'Aquino et al., 1996
Satsumas 'Okitsu'	Yang PT 65	13.1%	9.1%	Satsuma fruit segments wrapped with Yang PT 65 film had higher vitamin C content	Piga et al. (2002)
'Mosambi' sweet orange	Cryovac heat-shrinkable film	Not reported		Fruit trays wrapped with Cryovac heat-shrinkable film had higher vitamin C content compared to LDPE or HDPE films	Ladaniya and Singh (2001)
'Minneola' tangelo	Cryovac MR 15	14%	6.2%	Vitamin C content was not significantly different in fruit wrapped with Cryovac MR 15, Coop box CX 15, Cryovac PY 85 or unwrapped fruit	D'Aquino et al., 1998

Three levels of genome research

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Plant Physiology
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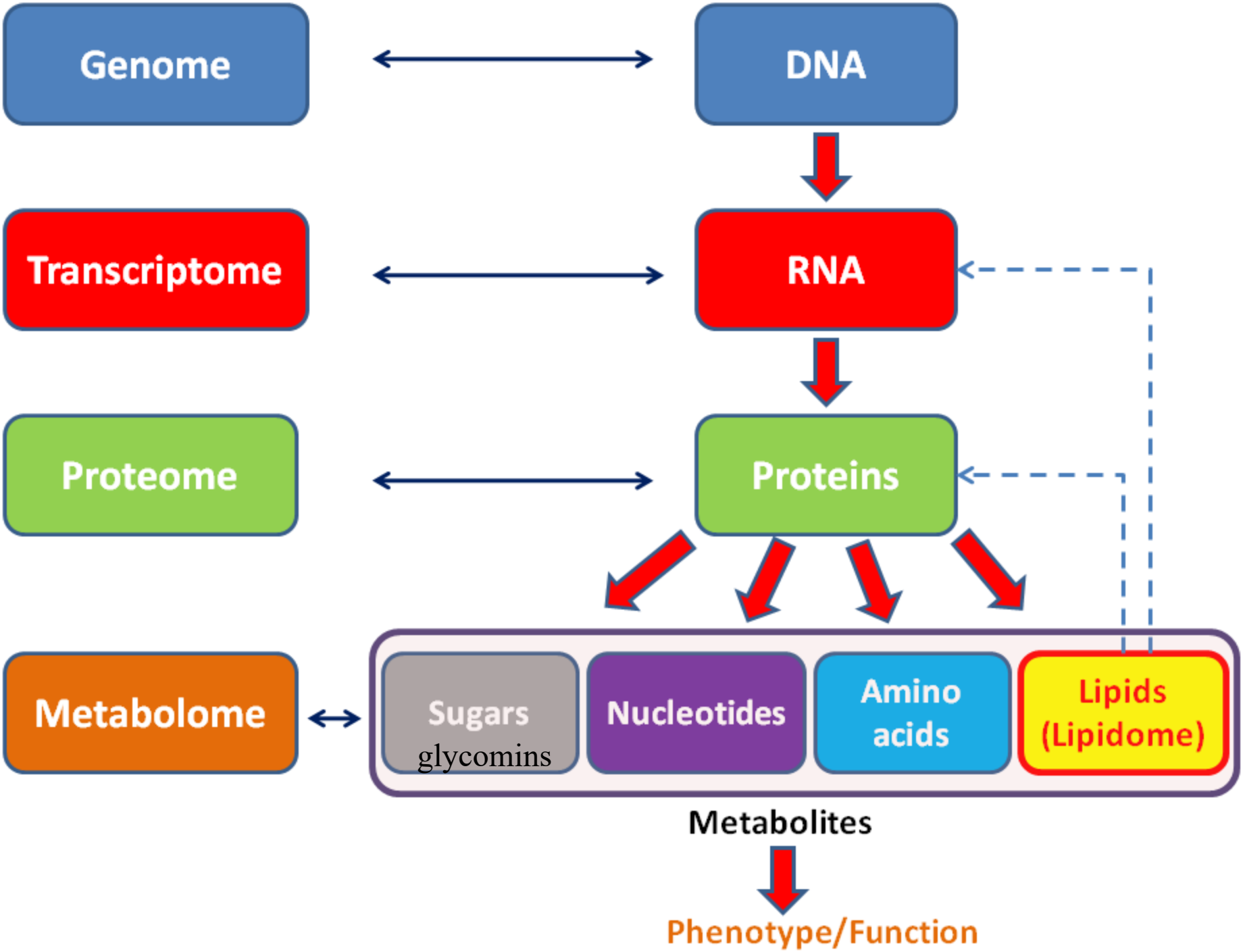
تحقیقات فیزیولوژِی


Genome
DNA
Transcriptome
RNA
Proteome
Proteins
Metabolome
Sugars
glycomins
Nucleotides
Amino acids
Lipids (Lipidome)
Metabolites
Phenotype/Function

Structural genomics

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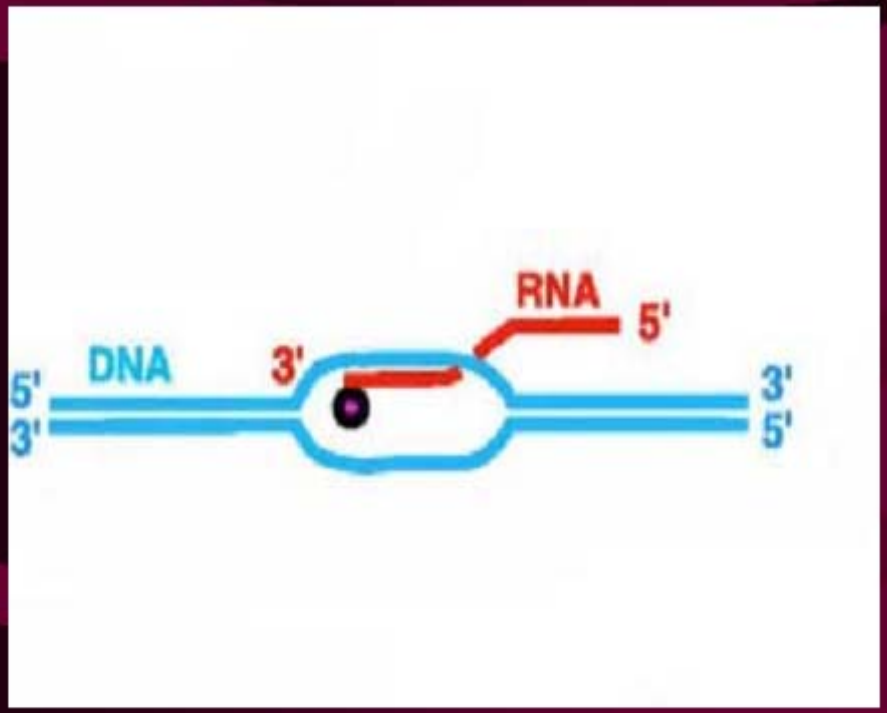
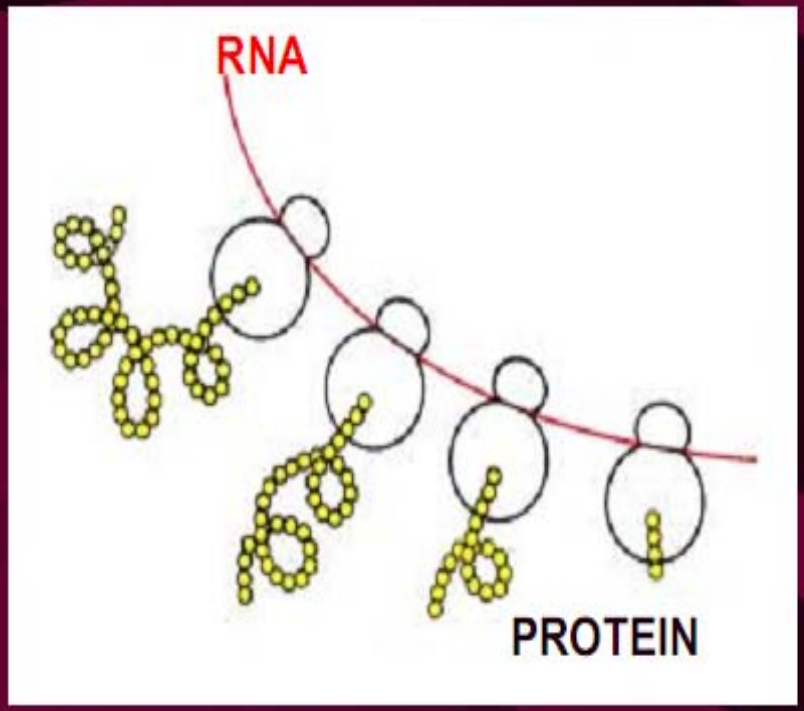
- cDNA
- Real Time PCR
- Microarray

Functional genomics

- مطالعات بعد از DNA





GENOME

TRANSCRIPTION

TRANSCRIPTOME

TRANSLATION

PROTEOME

مسیر شناسایی ژن تا اصلاح نباتات:

1- شناسایی

2- تایید

3- استفاده در اصلاح نباتات

تغییر بیان ژنها در شرایط تنش:

1- ژن های رمز کننده پروتئینهای با وظایف ساختاری یا آنزیمی مشخص

2- ژن های رمز کننده پروتئینهای با وظایف شناخته نشده

3- ژن های رمز کننده پروتئینهای تنظیمی

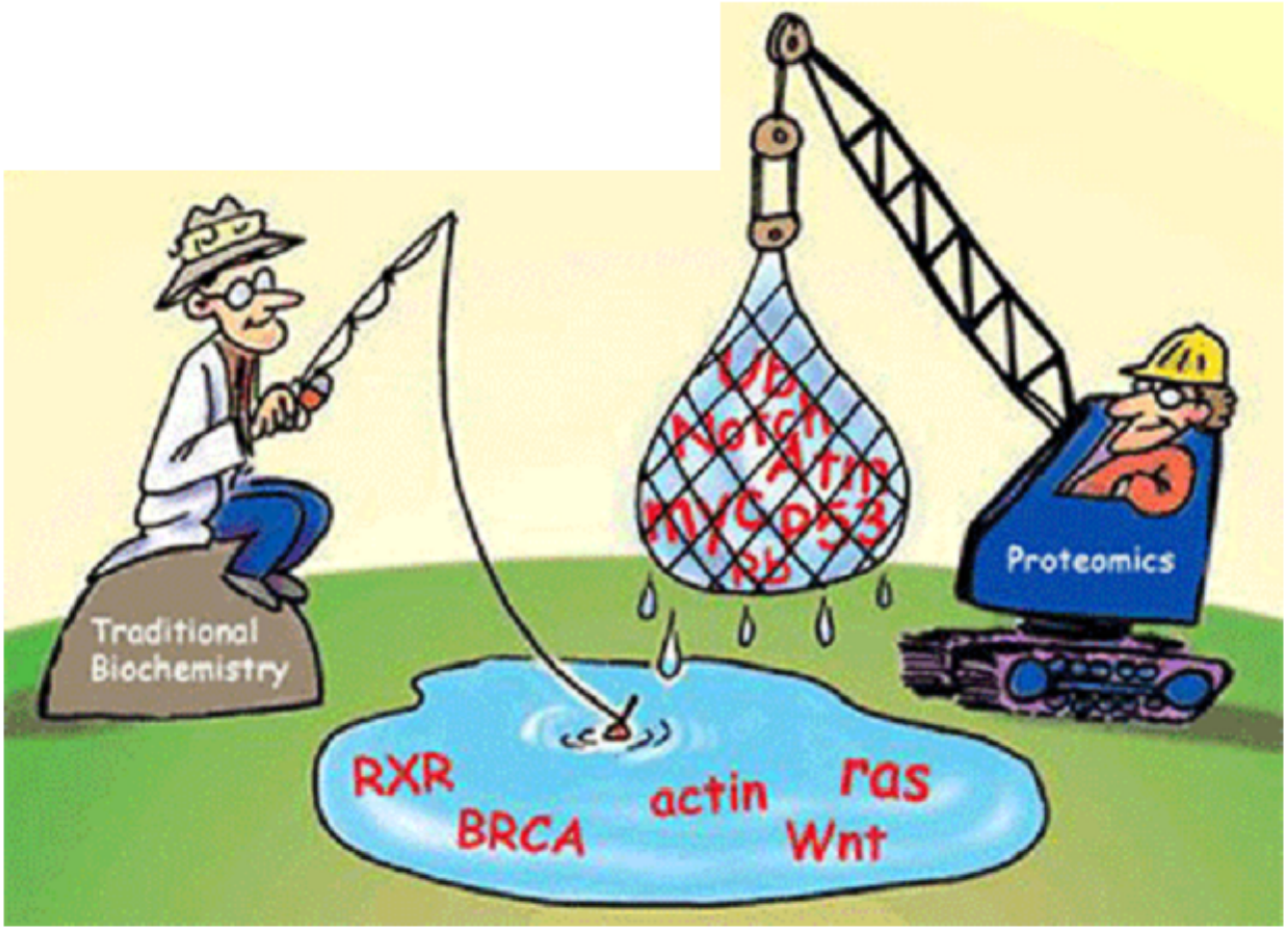
اگرچه انتخاب بر اساس صفات مرفولوژیک و فیزیولوژیک اهمیت بسیاری دارد، اما در مورد صفات پیچیده ای مانند مقاومت به شوری و خشکی، برای غلبه بر پیچیدگی مکانیسم ها به استفاده از مارکرهای مولکولی نیاز است.

امروزه تلاش زیادی در زمینه نقشه یابی جایگاه های کنترل کننده صفات کمی یا QTL (Quantitative Trait Loci) انجام میشود، تا با استفاده از آنها کارایی و سرعت انتخاب به کمک مارکرها (Marker Assisted selection) را افزایش داد.

در حال حاضر به دلیل عدم شناخت از ژنهای دخیل در پاسخ به تنش، استفاده از مارکرها با محدودیت هایی همراه است.

- مکانیسم مولکولی رفتار سلولها در شرایط مختلف را نمی توان از روی توالی ژنهای آنها پیشگویی کرد.
- رفتار سلول و تمام فعالیت هایی که در سلول انجام میشود بر عهده پروتئین ها است.
- عرصه های جدید پروتئومیکس با قابلیت ایجاد حجم بسیار زیاد اطلاعات، در ترکیب با روشهای نوین ژئومیکس و بیوانفورماتیک، امکان شناسایی مکانیسم ها و ژنهای دخیل در پاسخ به تنشها را فراهم میکند.
- در صورت استفاده از توان بالای تفکیک پروتئینها، انواع تغییرات کیفی و کمی پروتئین در شرایط تنش و بدون تنش در گیاه مقاوم و حساس مطالعه میشود.

Proteomics


Traditional Biochemistry
Proteomics
RXR
BRCA
actin
ras
Wnt

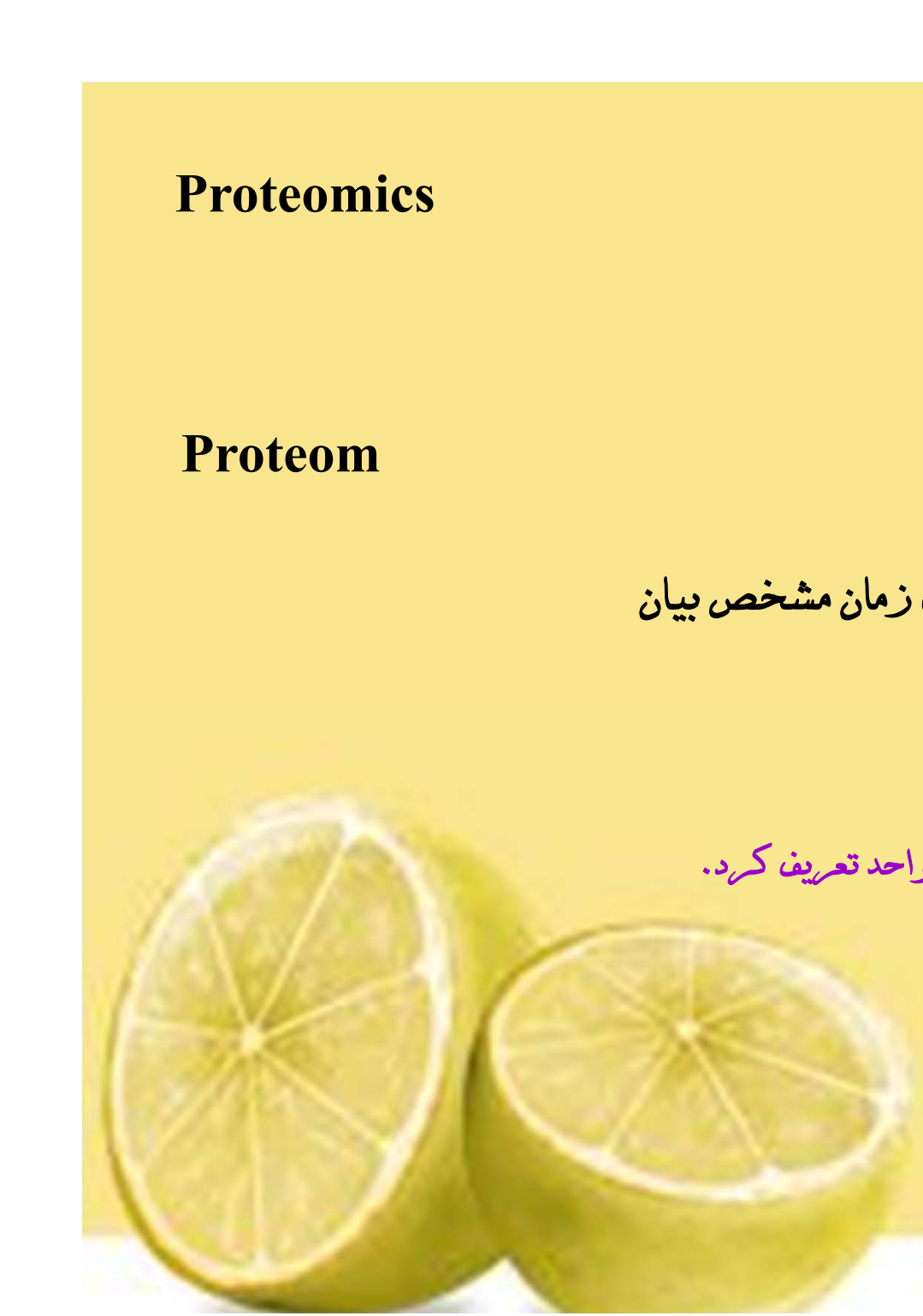
Proteomics

دانش بررسی ساختار و عملکرد پروتئینها

Proteom

به کلیه پروتئینهایی که در یک سلول در یک زمان مشخص بیان میشوند، پروتئوم آن سلول گفته میشود.

بر خلاف ژنوم برای هر موجود نمیتوان یک پروتئوم واحد تعریف کرد.
پروتئوم سلولهای مختلف با یکدیگر متفاوتند.
پروتئوم یک نوع سلول نیز همیشه ثابت نیست.



دانش بررسی تغییرات پروتئین در شرایط مختلف، عملکرد آنها و برهمکنش بین پروتئین های مختلف

30,000 تا 50،000 gene
15،000 تا 10،000 mRNA
But:
10,000 to 150,000 pr

- The complete set of proteins found in each cell is known as the proteome
- Approximately 25,000 proteins in a plant cell
- Proteins concentration (and activity) may be different than gene expression due to post-translational modification

GOALs

مشخص کردن کلیه پروتئینهایی که در سلول تحت یک شرایط معین بیان میشوند یا میزان بیان آنها تغییر میکند

شناسایی برهمکنش های بین پروتئینی (مسیرهای انتقال سیگنال و مسیرهای بیوسنتزی)

نقشه برداری آرایش های پروتئینی (آرایشهای پس از ترجمه مانند گلیکوزیله شدن، متیله شدن، فسفریله شدن و ...)، که بر فعالیت، عملکرد، ساختار فضایی، پایداری و نیمه عمر آنها اثر دارد

JOURNAL OF PROTEOMICS 74 (2011) 1301–1322

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Review

Plant proteome changes under abiotic stress — Contribution of proteomics studies to understanding plant stress response

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دو استراتژی اصلی گیاه در مقابله با تنش:

اجتناب از تنش

تحمل به تنش (تنظیمات قابل برگشت فعال در شرایط تنش) که معمولا **acclimation** نامیده میشود.

خوگیری به تنش از طریق تغییر در بیان ژنها صورت میگیرد که نتیجه آن تغییرات در ترانسکریپتوم، پروتئوم و متابولوم گیاه است.

نقش پروتئینها در خوگیری به تنش:

تغییر در غشای پلاسمایی
آنزیم های کاتالیز کننده تغییرات در سطح متابولیتها
تغییر در ساختار و میل ترکیبی ستوپلاسم به آب
کنترل رونویسی و ترجمه

مطالعات پروتئومیکس میتواند در نهایت منجر به شناسایی مارکرهای پروتئینی بالقوه شود. طوری که تغییر در فراوانی پروتئین های خاص با تغییرات کمی در برخی پارامترهای فیزیولوژیک مهم در پاسخ به تنش همراه باشد.

Plant response to stress

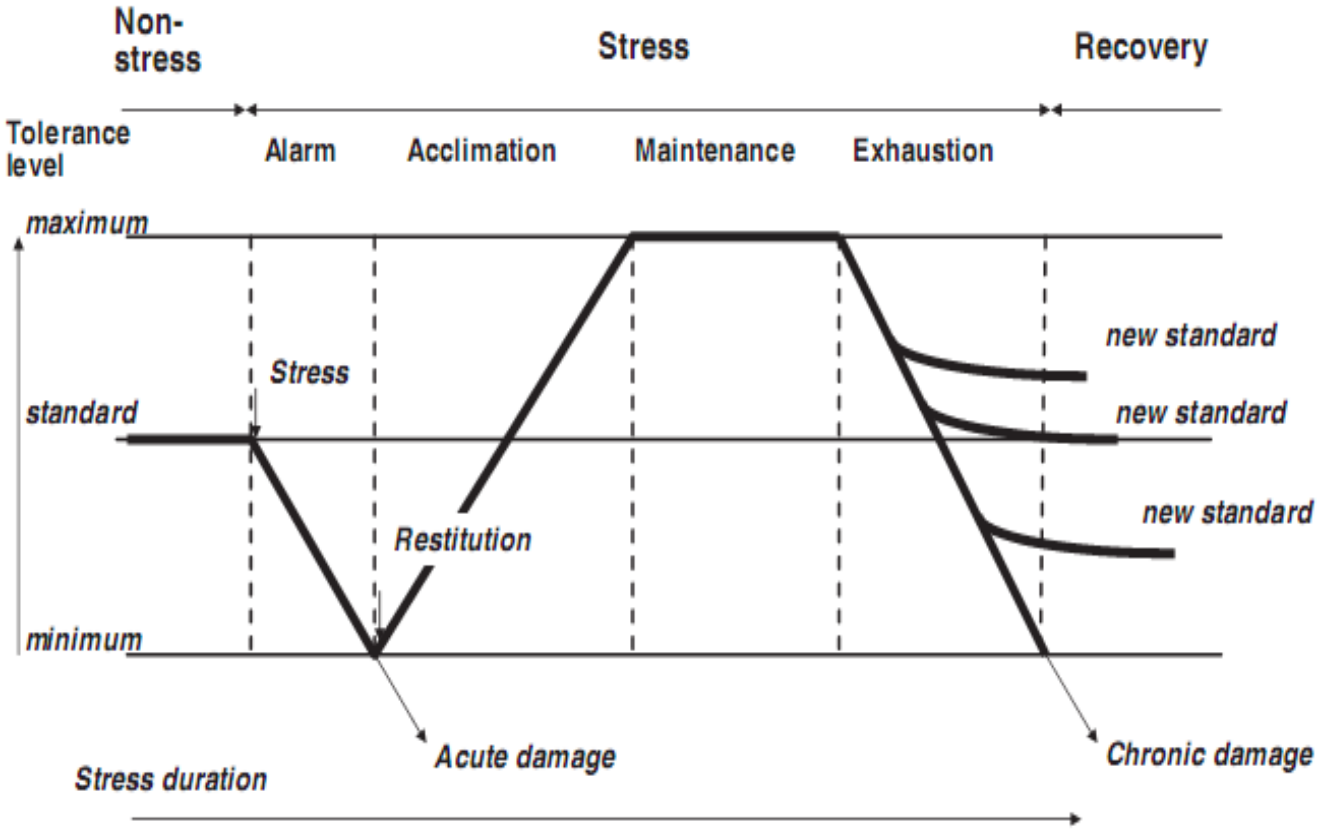


Fig. 1 – A generalised scheme of a dynamics of plant response to abiotic stress factors (modified after [2]). Each stage of plant stress response corresponds to a different proteome composition. Non-stressed plants reveal active growth and developmental progress based on cell division. These processes are associated with a *de novo* biosynthesis of several cellular components. In stressed plants, a profound reorganisation of the whole cellular metabolism is observed. There is a shift from an active growth and developmental progress to stress acclimation. Early stages of plant stress response (alarm phase) are associated with an induction of stress-responsive signalling pathways and a strong oxidative stress. Later stages (acclimation phase) are associated with a *de novo* biosynthesis of several stress-protective proteins (e.g., chaperones, COR/LEA, PCs, ROS scavenging enzymes) and other compounds (e.g., antioxidants — carotenoids, tocopherols; osmoprotectants — GB, proline). During recovery, processes leading to degradation of stress-protective compounds are activated and a new cellular homeostasis is being established.

خوگیری، فرایند فعالی است که به انرژی نیاز دارد. متابولیسم گیاه از مسیر رشد و نمو فعال سابق به سمت خوگیری به تنش تغییر میکند که این تغییرات در سطح پروتئوم انعکاس می یابد

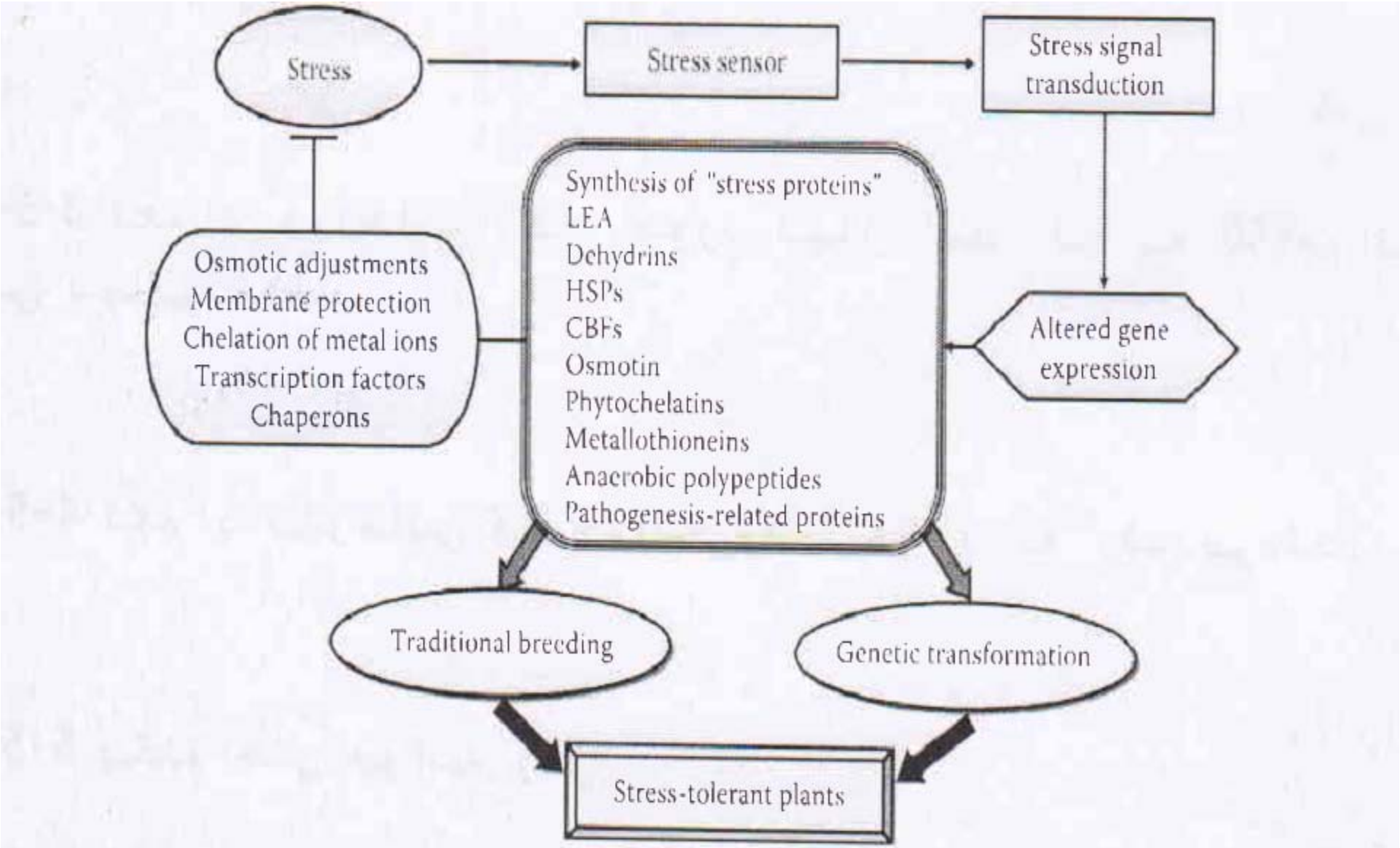


FIGURE 19.1 Stress-induced protein synthesis in plants. Stresses cause important modifications in the gene expression in plants, which leads to the synthesis and accumulation of stress-related proteins. These proteins provide enhanced survival value to plants under adverse environmental situations and can be used to produce stress-tolerant plants by genetic transformations. For details, refer Section 19.2.

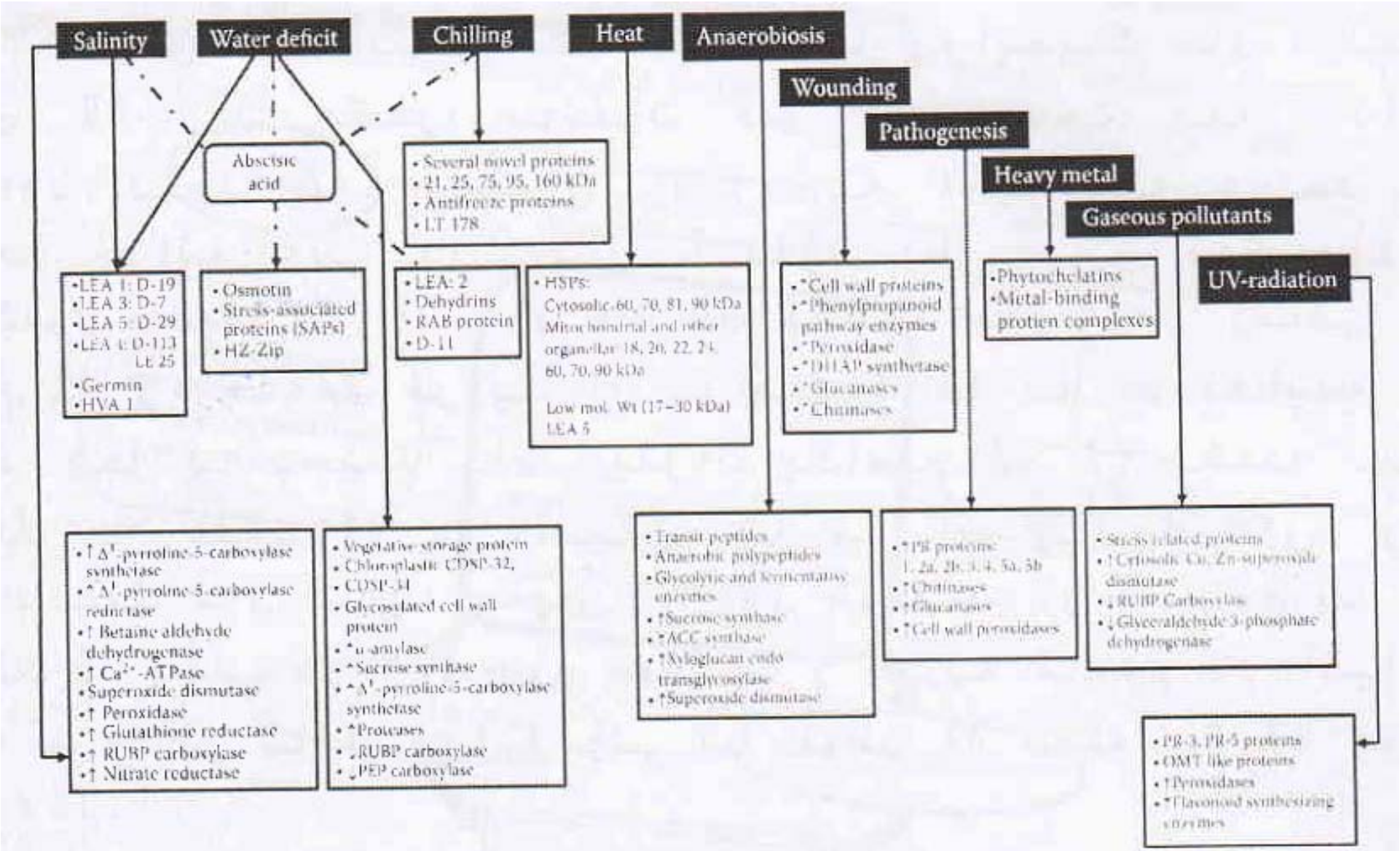
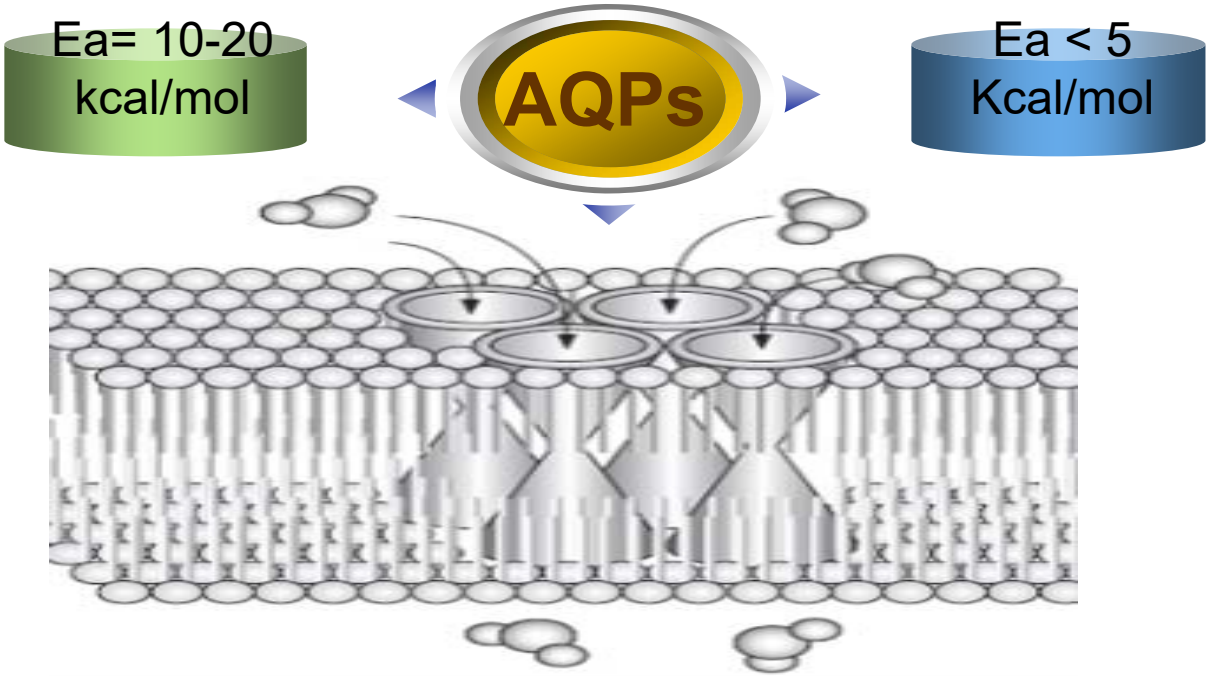


FIGURE 19.2 An overview of stress-induced protein-synthetic responses in plants. Different stresses induce the synthesis of various groups of proteins and cause either elevation (↑) or decline (↓) in the levels of enzymes. Some of the responses of salinity, drought, and chilling are common and are mediated via elevated levels of ABA. For details, refer Section 19.1.

Aquaporins (AQPs)



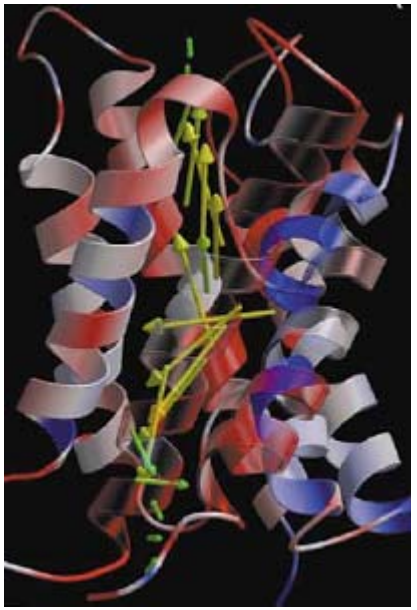
Two main topics were of special interest

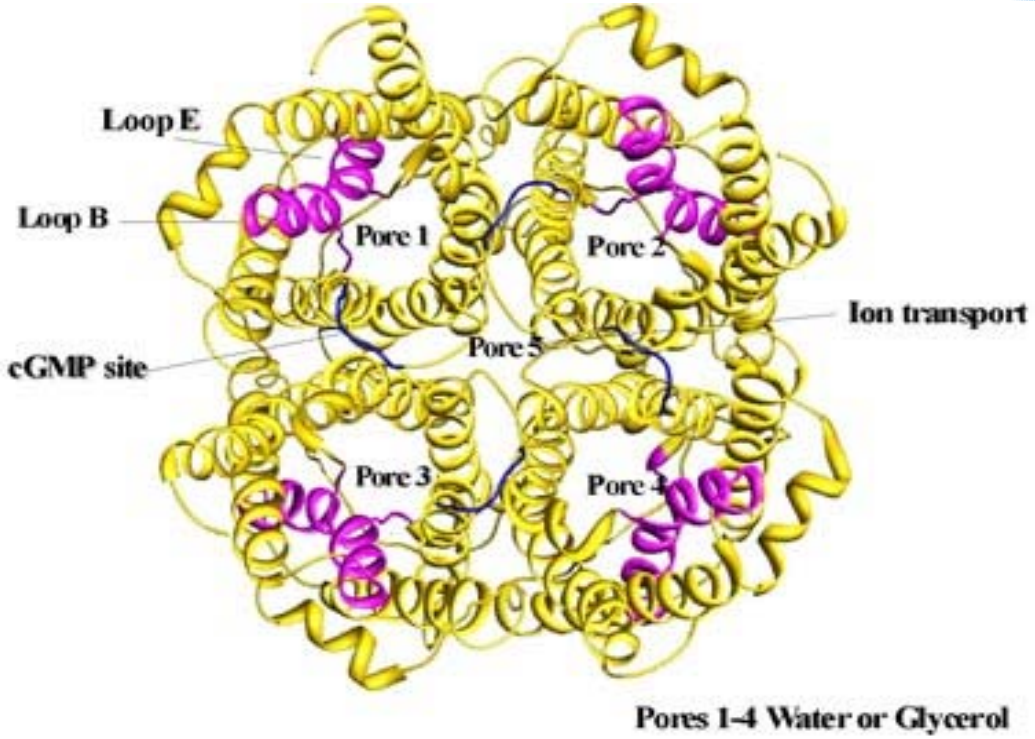
◇The high water transport rate (10^9 molecules per second)

◇The high selectivity for water

AQPs subfamilies:

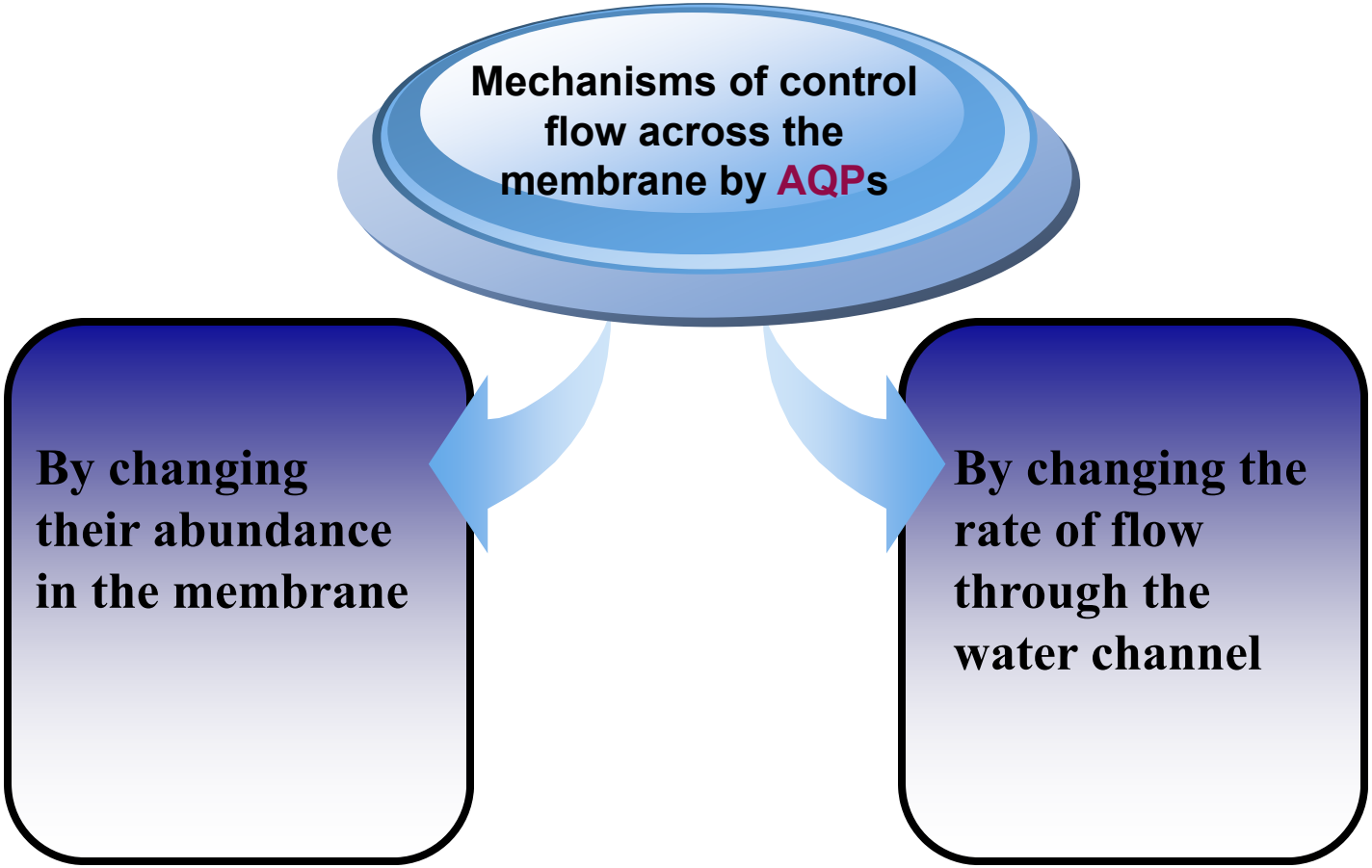
- Plasma membrane intrinsic proteins (PIP)
- Tonoplast intrinsic proteins (TIP)
- Nodulin26-like intrinsic proteins (NIP)
- Small basic intrinsic proteins (SIP)



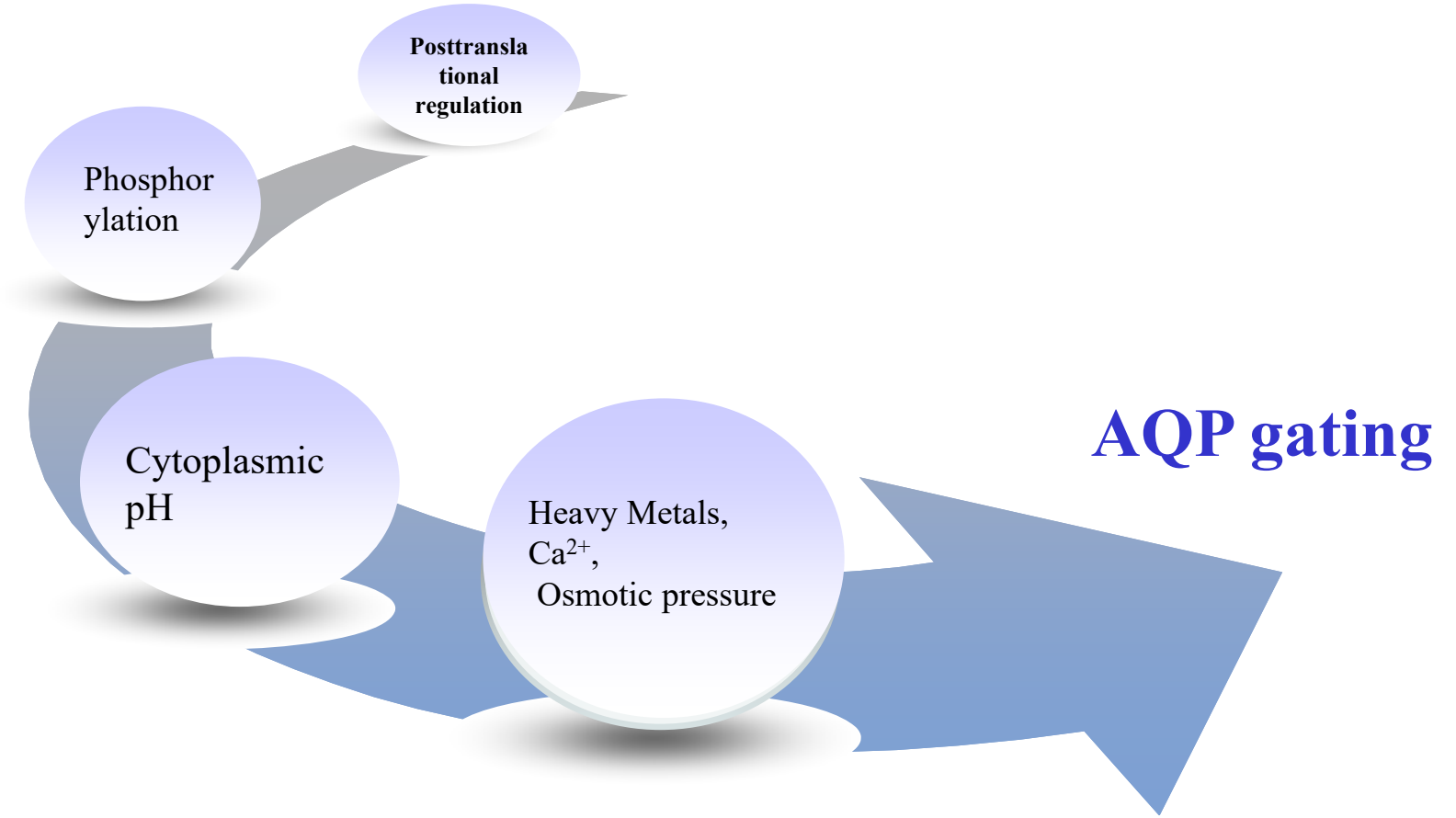


In the membrane, AQPs form tetrameric structures with each monomer acting as an independent water channel

Control of water permeability



Change in the rate of water flow



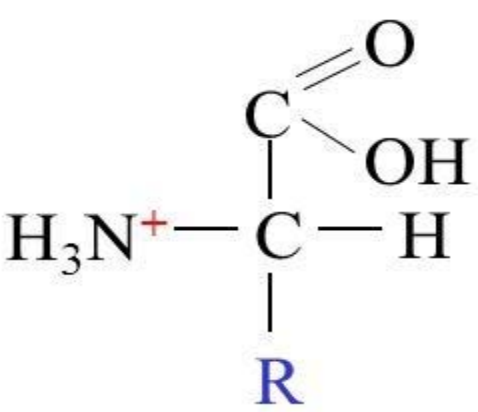
Separating the Proteome

- The protein genome is separated by several different methods.

- The most commonly used method is 2-dimensional gel electrophoresis.
  - Consists of using isoelectric focusing with SDS polyacrylamide gel electrophoresis

Isoelectic point

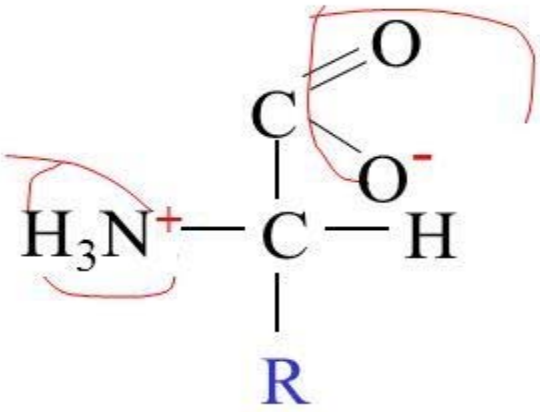
acid-base chemistry



low pH

amine and c.a. protonated

positive charge

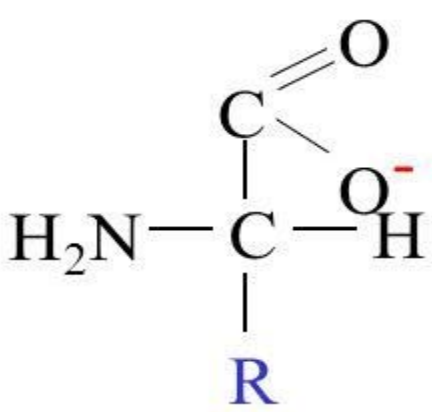


neutral pH

amine protonated

c.a. deprotonated

no net charge



high pH

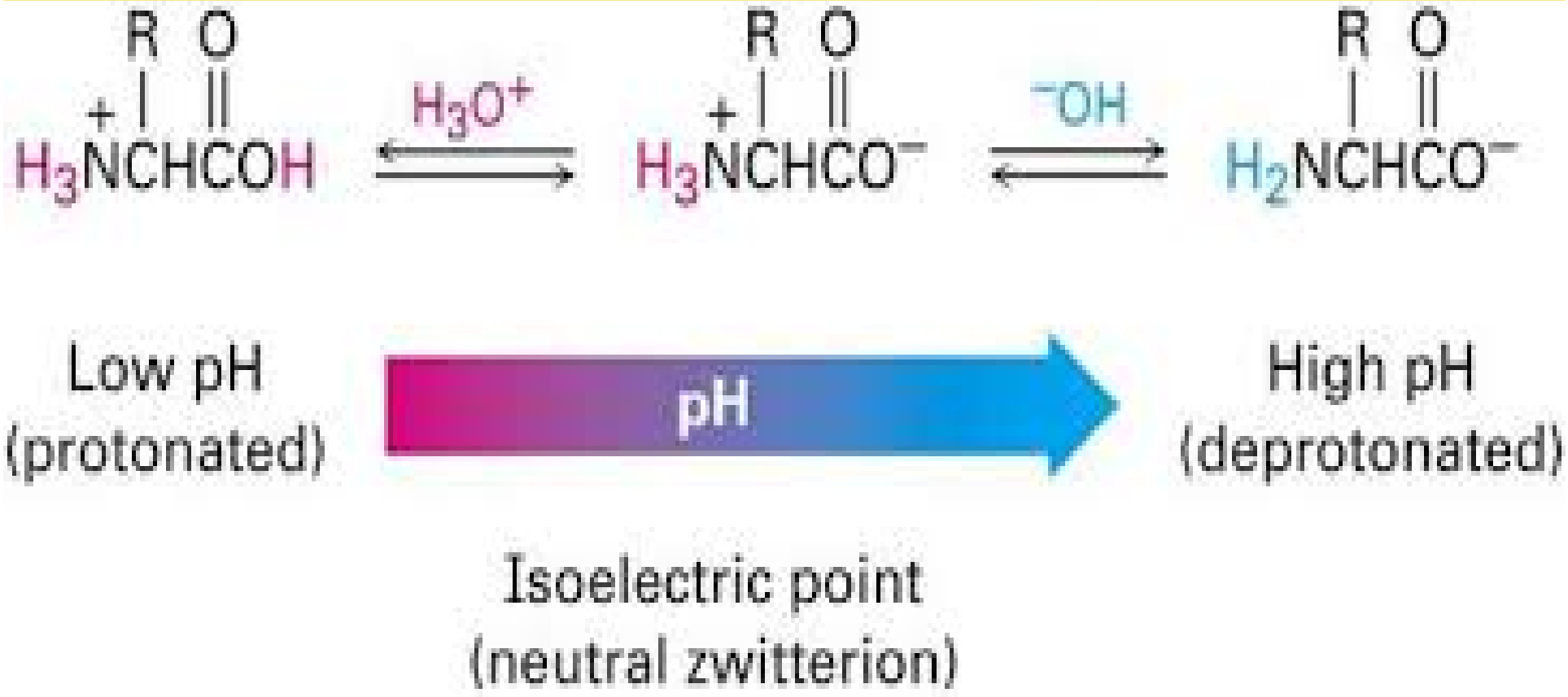
amine and c.a. deprotonated

negative charge

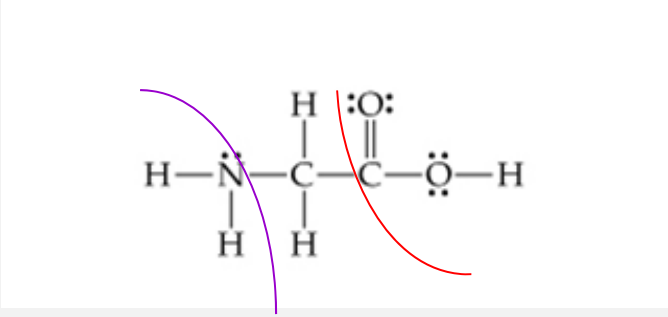
isoelectric point $pH = pH_I$

amino acids diprotic acids 2 pK_a

در یک pH خاص، بارهای + و _ برابر صفر است که این pH را pHایزوالکتریک می گویند.
هر پروتئین، pH ایزوالکتریک خاص خود را دارد که به اسیدهای آمینه آن بستگی دارد
در این نقطه، حلالیت پروتئین ها به حداقل می رسد که باعث رسوب می شود.

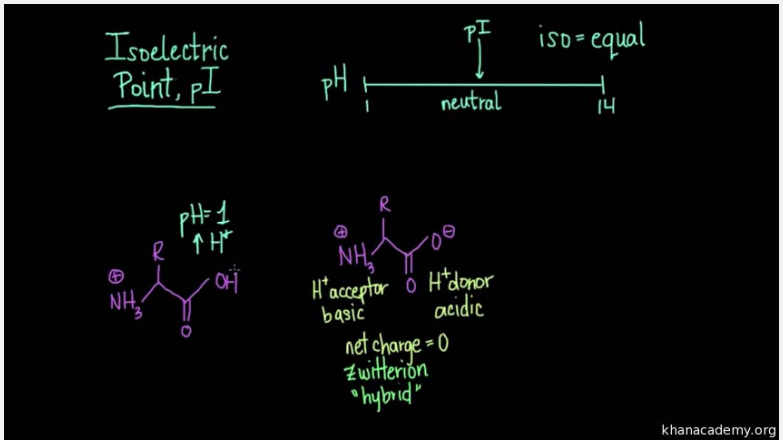


glycine



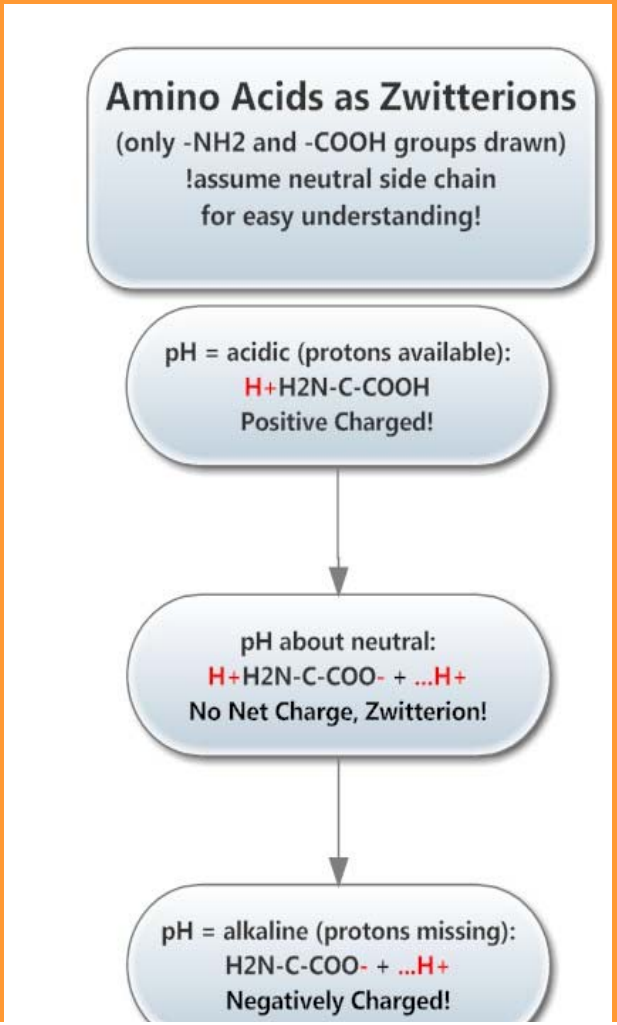
\+ -

در محیط خنثی بار خنثی
در محیط قلیایی بار منفی
در محیط اسیدی بار مثبت



اسیدهای آمینه غیرقطبی تقریبا در pH 7 به نقطه PI می رسند

اسیدهای آمینه باردار:
اسیدهای آمینه بازی در pHبازی به نقطه PI می رسند مثل لیزین، آرژنین، هیستیدین
اسیدهای آمینه اسیدی در pHاسیدی به نقطه PI می رسند مثل گلوتامیک اسید، آسپارتیک اسید



Isoelectric focusing

- This separates proteins based on isoelectric point
- The isoelectric point is the pH at which the protein has no net charge.

با اختلاط اسیدها و بازهای آلی دارای وزن مولکولی پایین و انتشار آنها در میدان الکتریک ایجاد شده در عرض ژل، یک شیب pH به وجود می آید.

وقتی مخلوط پروتئین در این شیب قرار داده می شود هر پروتئین برای رسیدن به pH برابر PI خود داخل ژل حرکت می کند

- pH gradients may be large 2-10 or small 6-7
- Typically this is done with an immobilized pH gradient gel strip or with a tube gel containing a low concentration of polyacrylamide.
- Ampholytes are added to create a pH gradient in an electric field and the proteins are loaded.
- The IEF gel is placed in an electrophoresis system for up to 24 hours and the proteins form tight bands at their isoelectric point.

الکتروفورز پلی اکریل آمید دو بعدی

1-immobilized pH gradient gel strip (IPG)

در ژل های IPG آمفولین های اسیدی در چپ، آمفولین های بازی در راست و آمفولین های خنثی در وسط در کارخانه با ژل میکس می شوند

برای جلوگیری از فیکس شدن پروتئین ها در دو سمت + و – ژل، به عصاره پروتئینی carrier ampholin اضافه می شود که خود بار داشته و با بار ژل رقابت می کنند.

Proteomics in ABRII

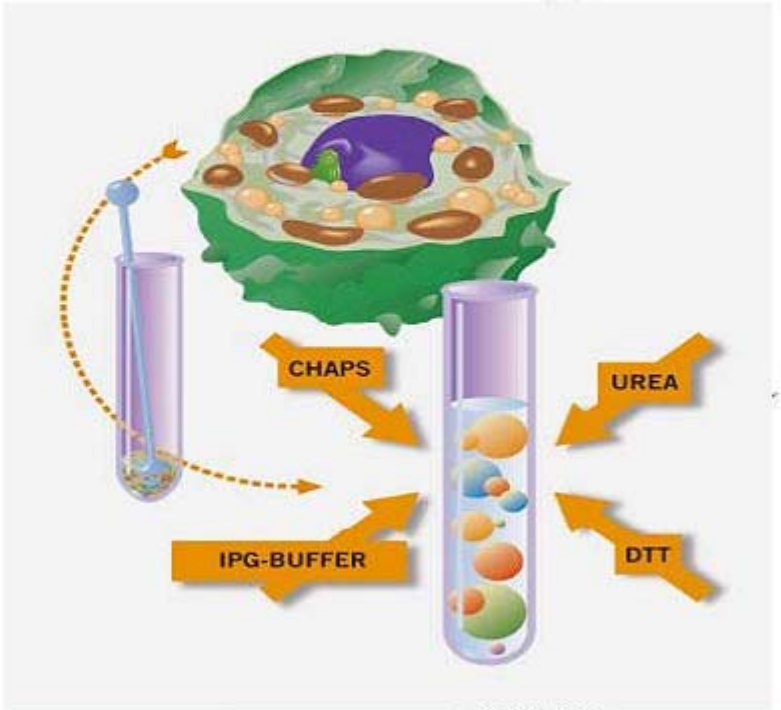
Sample preparation

↓

Protein extraction

↓

1st dimension (IEF)



TCA Acetone

Proteomics in ABRII

Sample preparation

↓

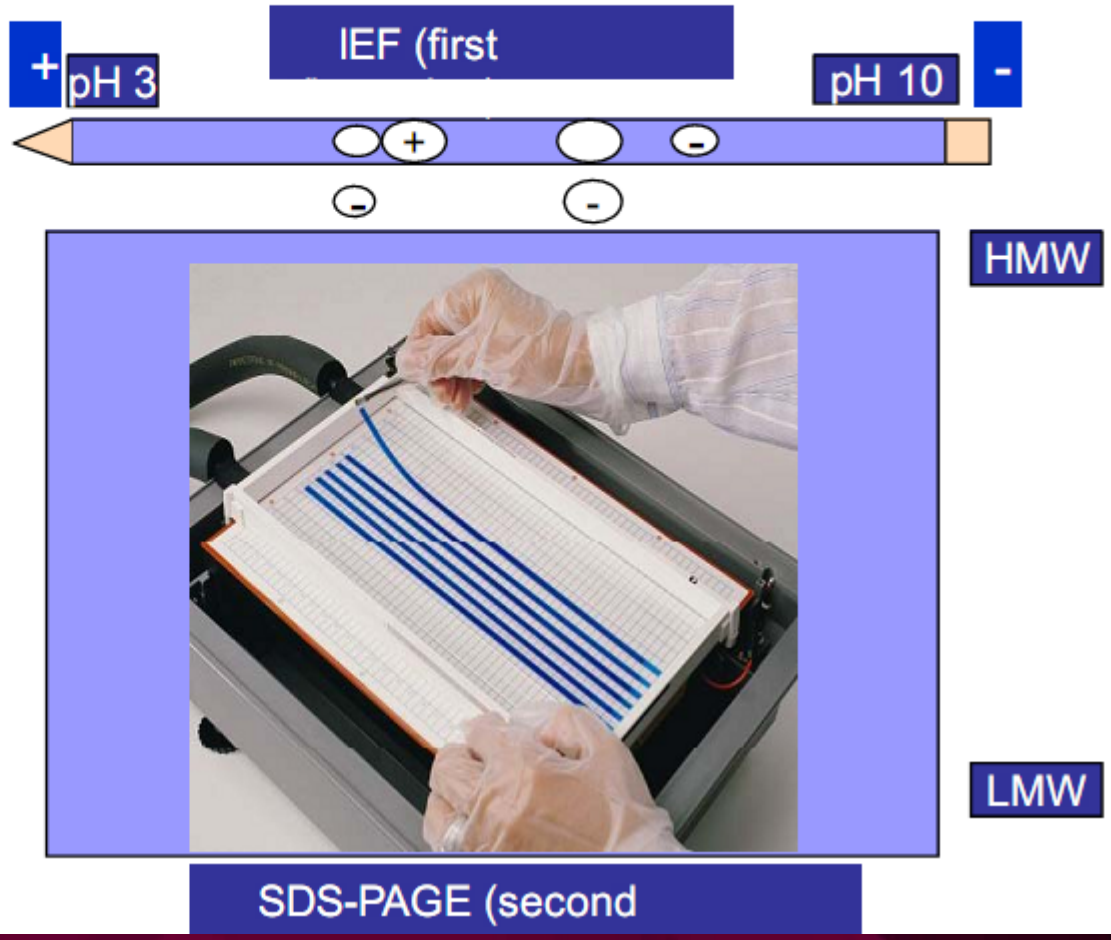
Protein extraction

↓

1st dimension (IEF)

↓

2nd dimension (SDS-PAGE)

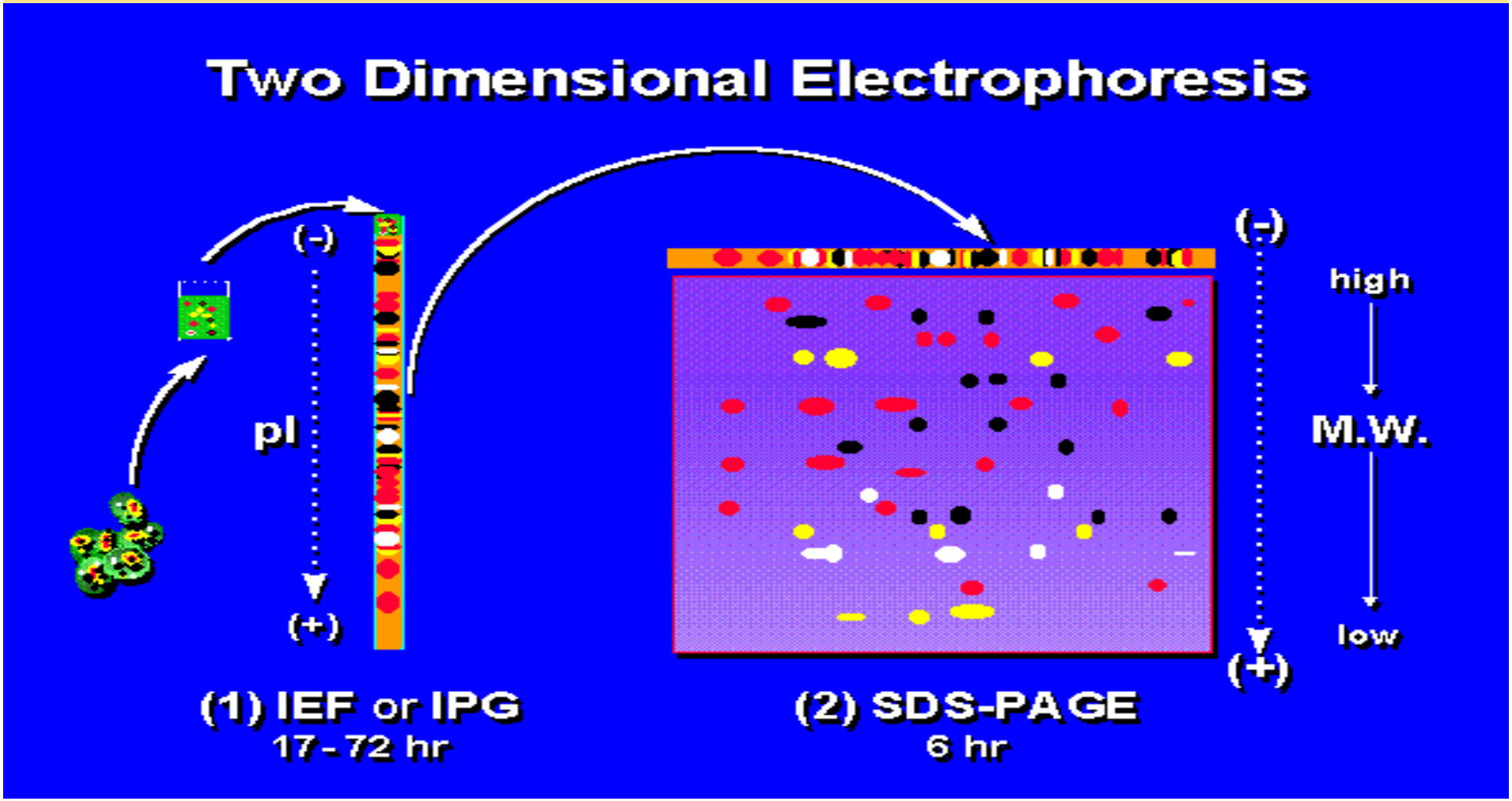


2- SDS Polyacrylamide Gel Electrophoresis

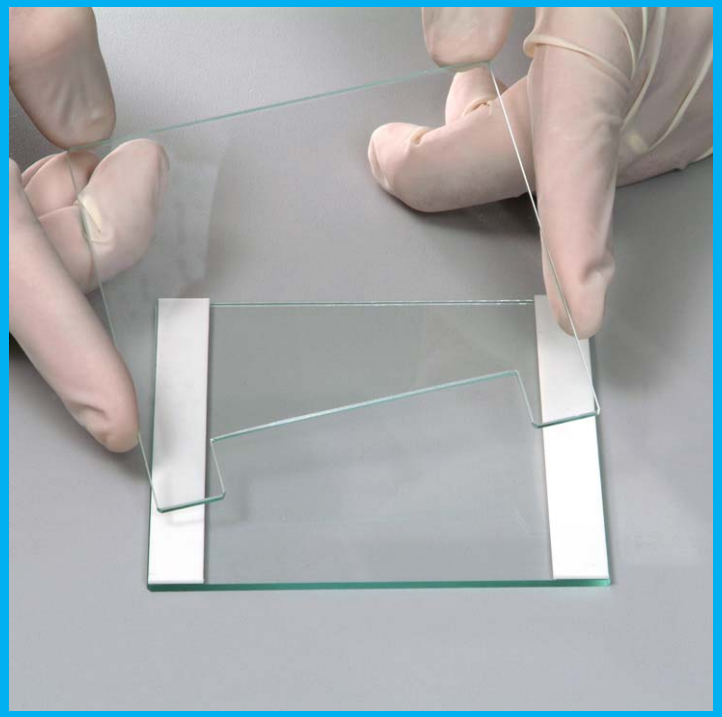
تکنیک SDS-PAGEبه دلیل استفاده از ماده سدیم دودسیل سولفات ((SDSو همچنین ویژگی های عالی ژل پلی اکریل آمید قدرت تفکیک پروتئین ها بسیار خوب می باشد. SDSیک دترجنت آنیونی می باشد که با اتصال به نواحی هیدروفوب پروتئین ها آنها را دناتوره می کند. در واقع مولکول SDSبا اتصال به پروتئین ها بار طبیعی آنها را می پوشاند و توزیع یکنواختی از بارهای منفی بر روی آن ایجاد می نماید. در نتیجه، جداسازی پروتئین ها تنها بر اساس وزن مولکولی‌شان صورت می گیرد. جهت خطی نمودن مولکول های پروتئینی، آنها را در مقدار کافی SDS، و ماده احیا کننده مرکاپتو اتانول جهت از بین بردن باندهای دی سولفیدی و نیز دقایقی حرارت قرار می دهند. مقدار SDSلازم جهت اتصال به پروتئین ها، ۱.۴ گرم SDSبه ازای هر گرم پروتئین می باشد. حالا در هنگام ران نمودن الکتروفورز جداسازی پروتئین ها تنها بر اساس وزن مولکولی شان خواهد بود. بدین معنا که هرچه اندازه مولکول بزرگتر باشد، حرکت آن به دلیل اصطکاک با محیط اطراف کمتر خواهد بود.

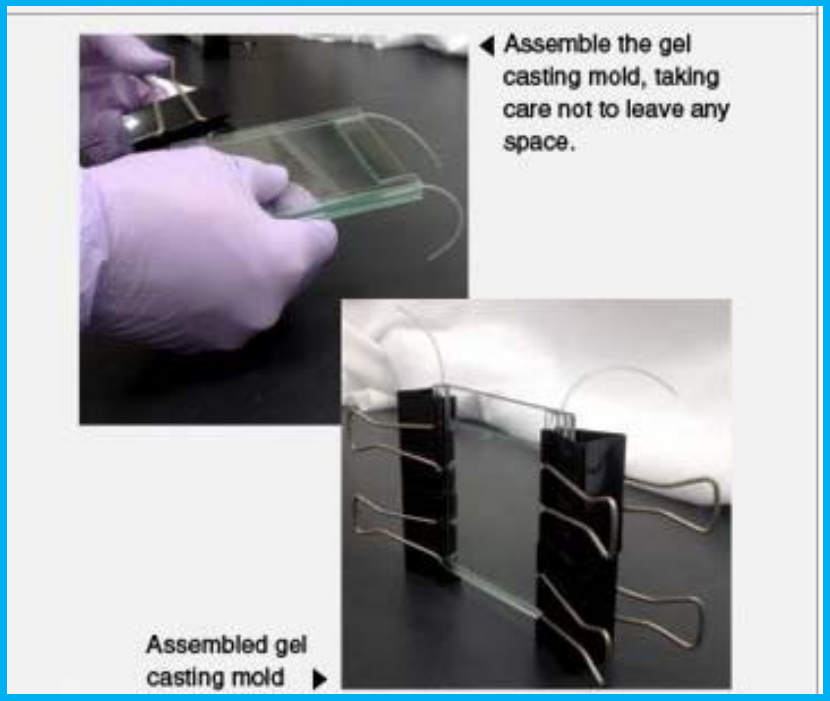
Thus, in 2D gel electrophoresis, the protein is allowed to run on a fixed pH gradient in the first dimension. In the second dimension, the proteins are separated using vertical or horizontal polyacrylamide gel electrophoresis. Thus, the proteins separate according to their molecular weight in the second dimension.

Besides, this method of gel electrophoresis increases the resolution of protein separation. Therefore, the separated proteins are purer. However, the cost of the technique is much higher than one dimension gel electrophoresis.



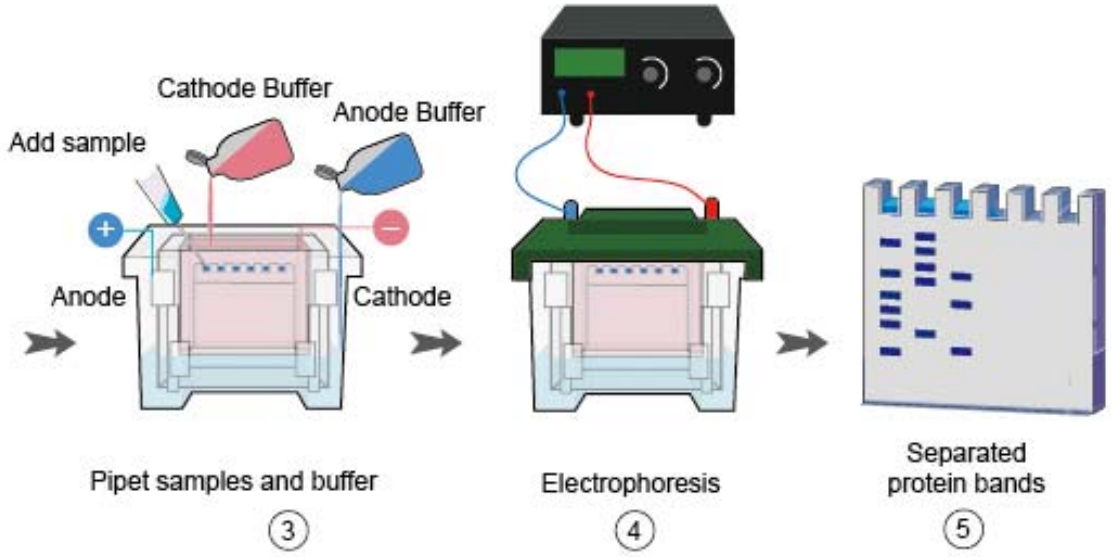
How To Cast and Run a Polyacrylamide Gel





تانک با دو نوع بافر با pH های متفاوت پر می شود:

Loading / tank buffer
Seperating / resolving buffer



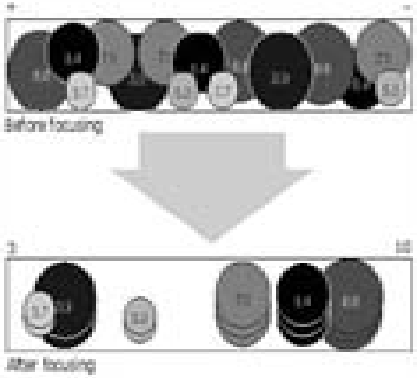
SDS Polyacrylamide Gel Electrophoresis

- The second dimension separates the proteins based on size.
- There are two parts, the stacking gel which concentrates the sample and the running gel that is used to separate the proteins.
- The IEF gel is soaked in a solution containing chemical to denature the proteins including sodium dodecyl sulfate a detergent which gives the proteins a net negative charge. This means that all proteins will move in one direction.
- The IEF gel is then put in the one long well in the stacking gel, sealed in place with agarose, and the proteins subjected to an electric field to separate.
- The larger proteins are found at the top and the smaller ones are found at the bottom of the gel.

Complete Extraction

Extract

Isoelectric focusing



SDS-PAGE

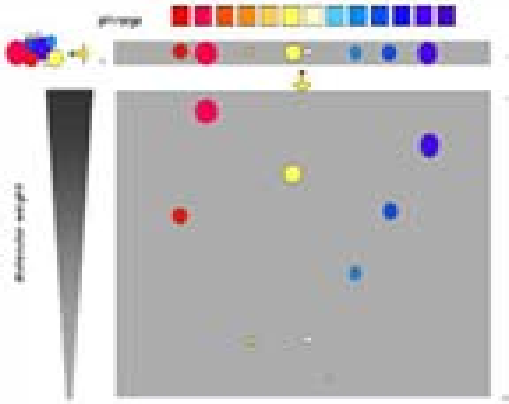
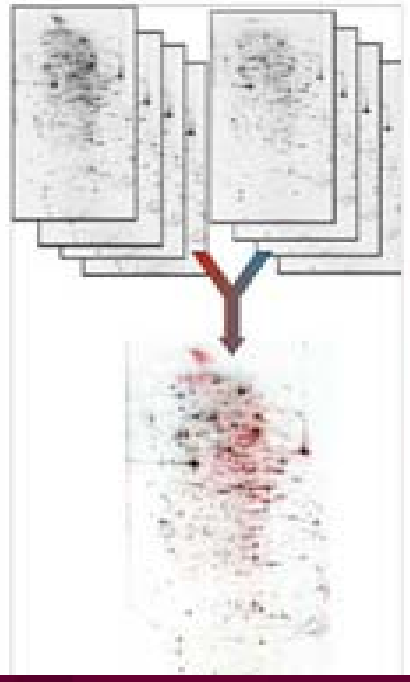
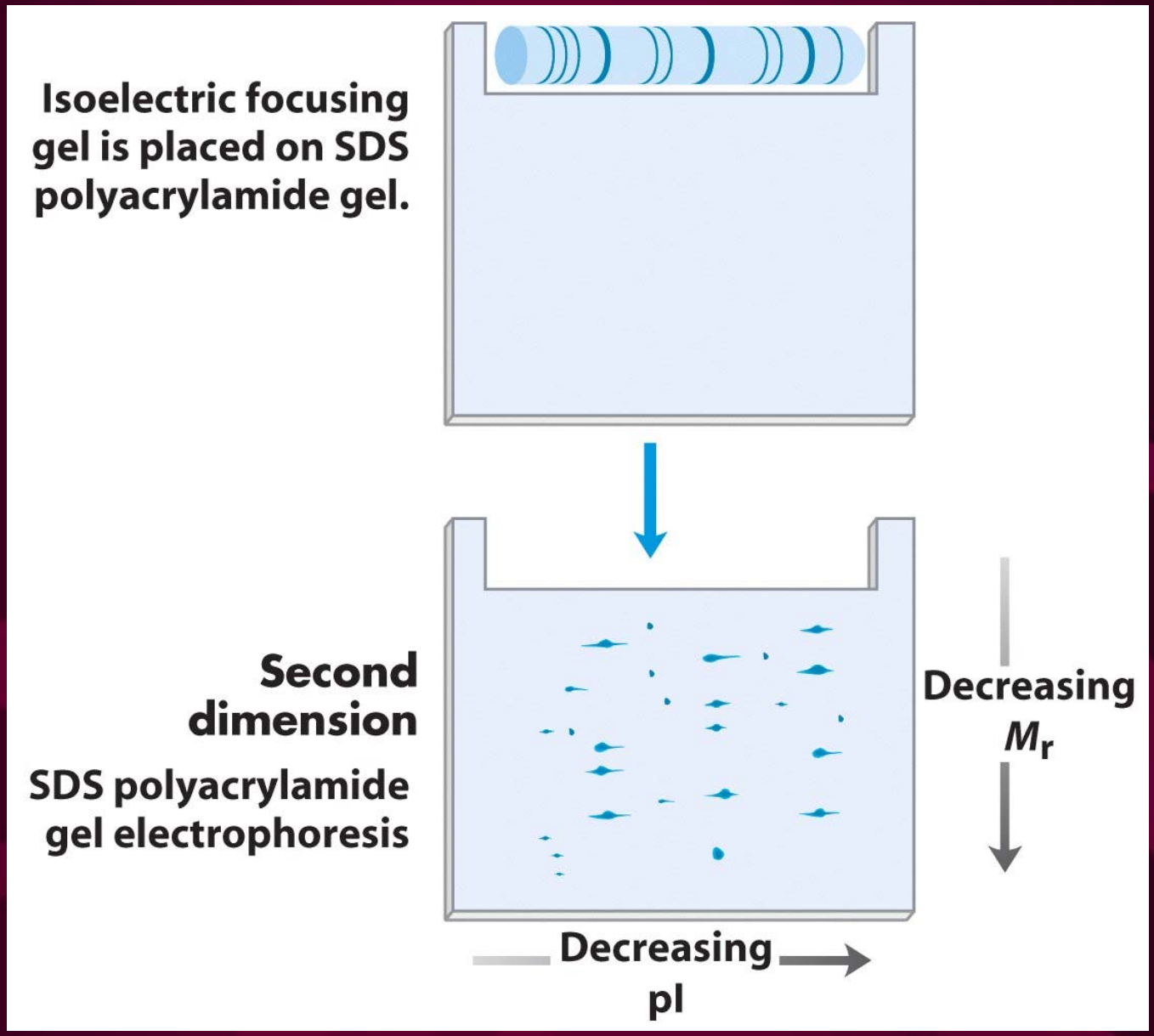


Image capture




Isoelectric focusing gel is placed on SDS polyacrylamide gel.
Second dimension
SDS polyacrylamide gel electrophoresis
Decreasing M_r
Decreasing pI

Proteomics in ABRII

Sample preparation

↓

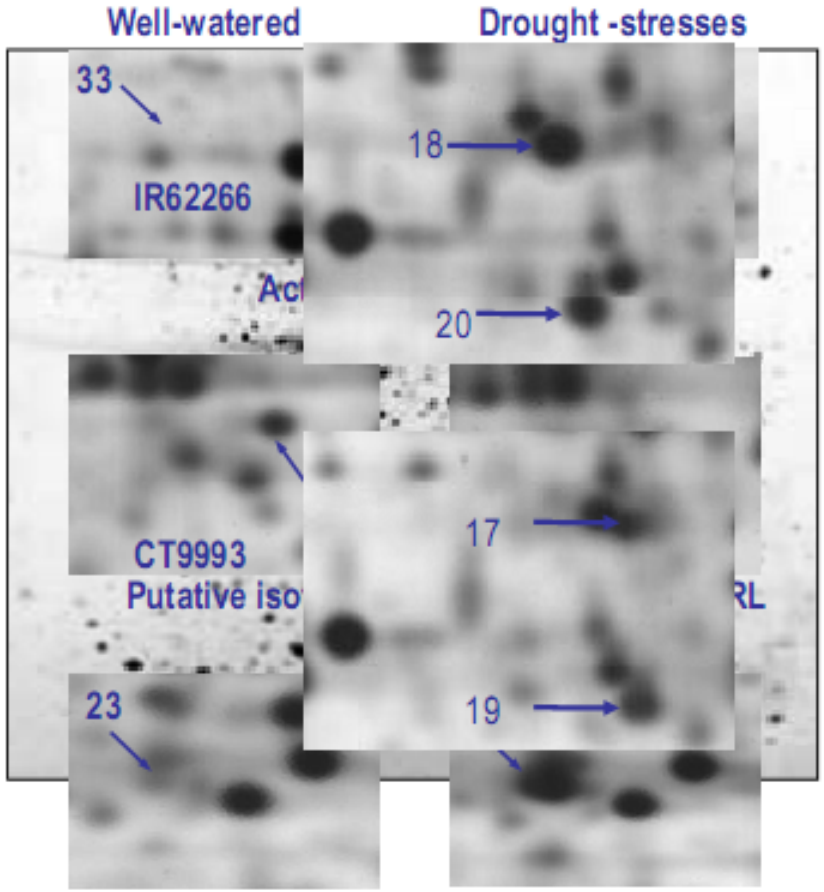
Protein extraction

↓

1st dimension (IEF)

↓

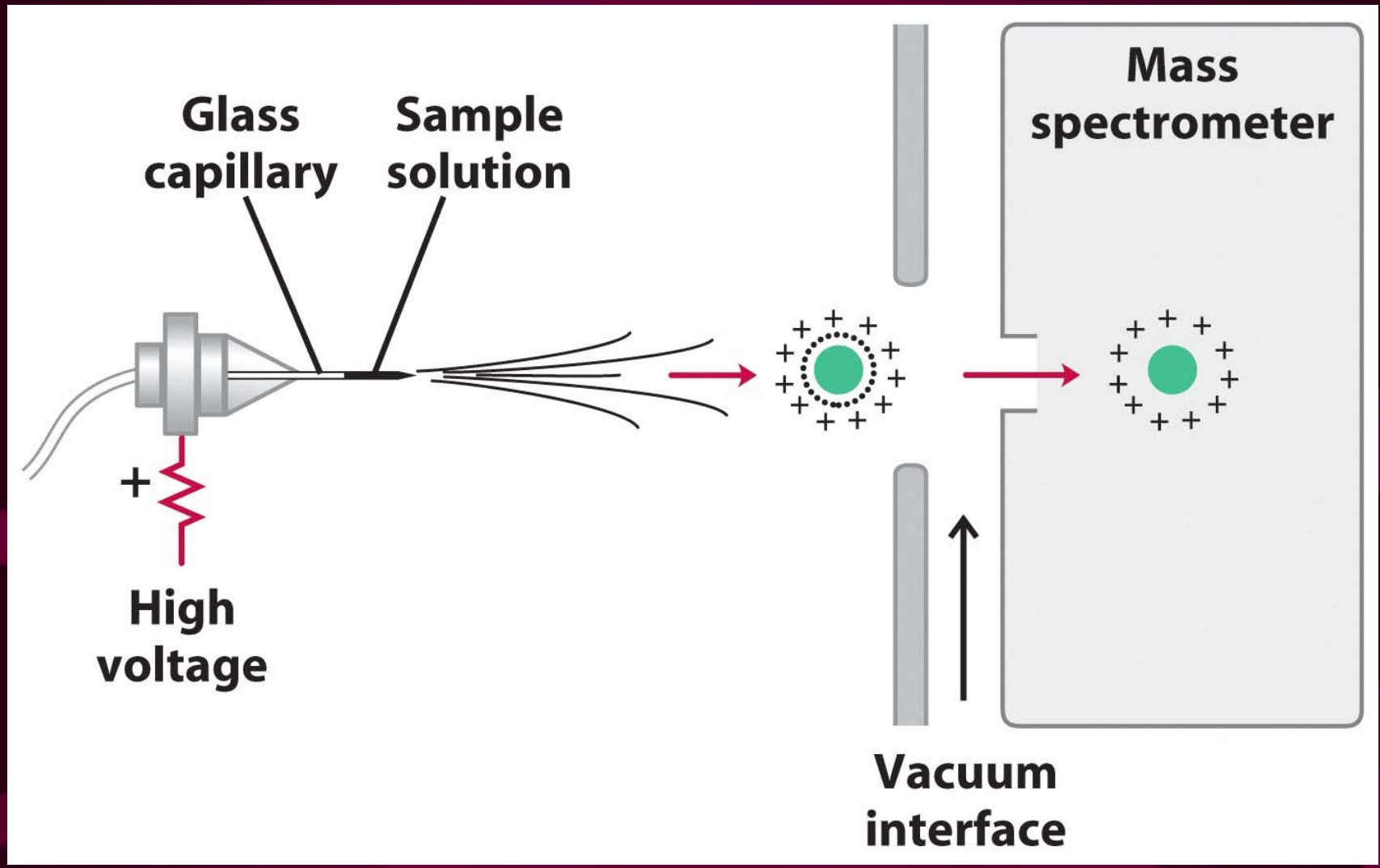
2nd dimension (SDS-PAGE)



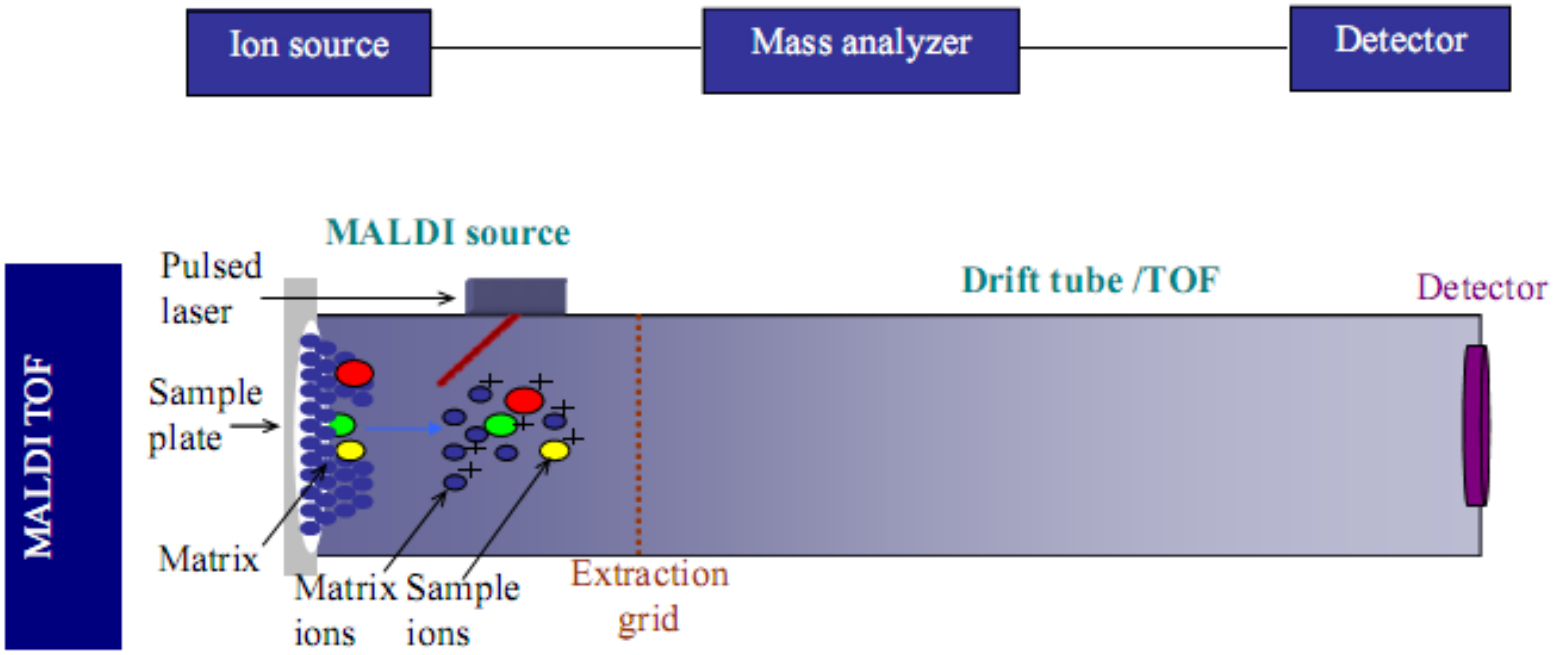
S-like RNase (RNase DIS)

Mass Spectrometery

- Separates ions based on mass to charge ratio.
  - Charges are placed on the protein or the peptide by ionization.
- Two most common types of ionization are:
- Matrix-Assisted Laser Desorption Ionization.
  - MALDI causes fragmentation of the protein during ionization. Can be used to get more information about the fragments. Easier to do than ESI.
- Electrospray ionization (ESI)
  - ESI can give whole protein masses as well as complex masses. If the proteins is first separated by reverse phase HPLC before injection only the subunits masses will be known.

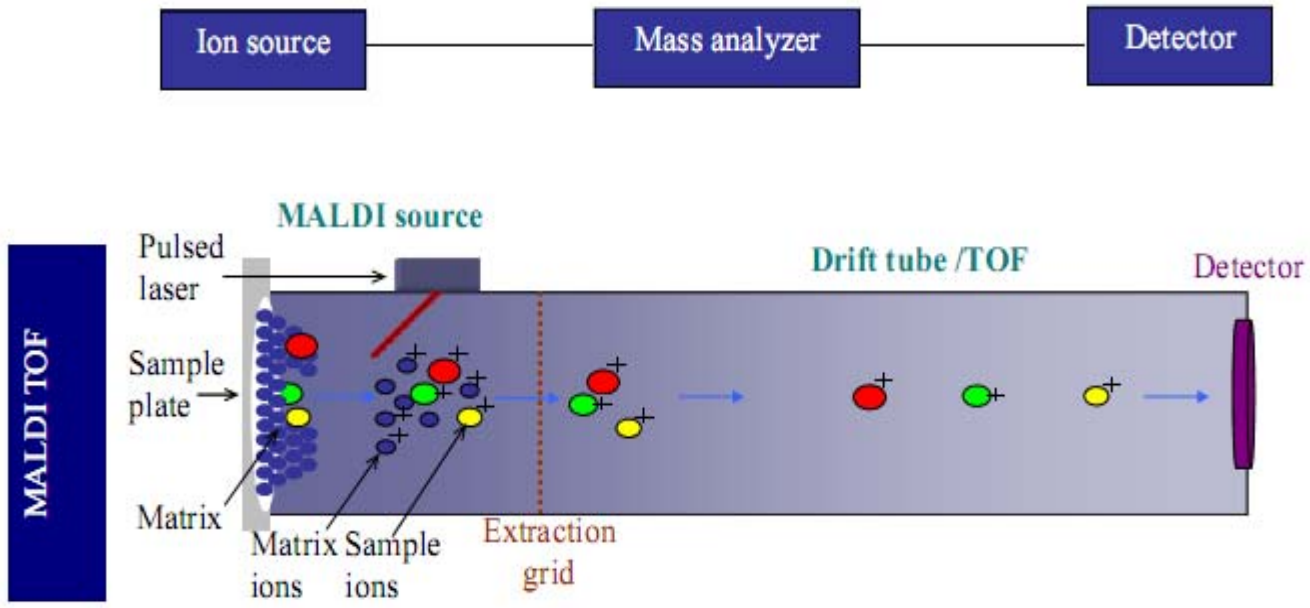

Glass capillary
Sample solution
Mass spectrometer
+
High voltage
Vacuum interface

Protein Identification Using Mass Spectrometry

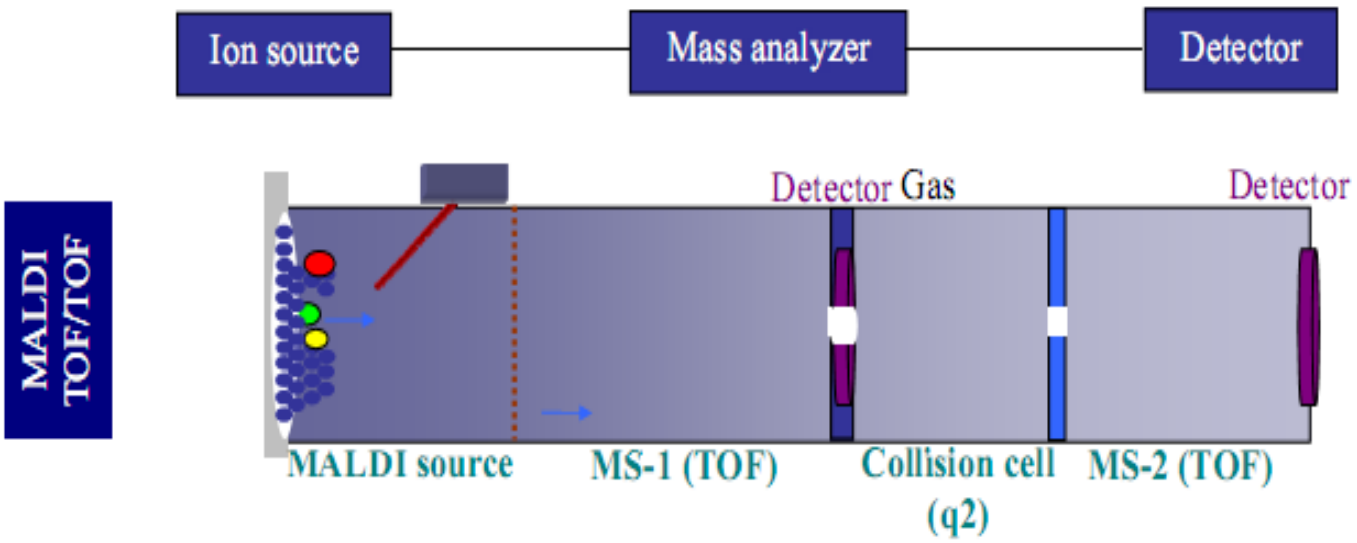


Baharvand, Fathi, VanHoof, Hosseini Salekdeh, 2007. ***Stem Cells,*** *25: 1888-1903.*

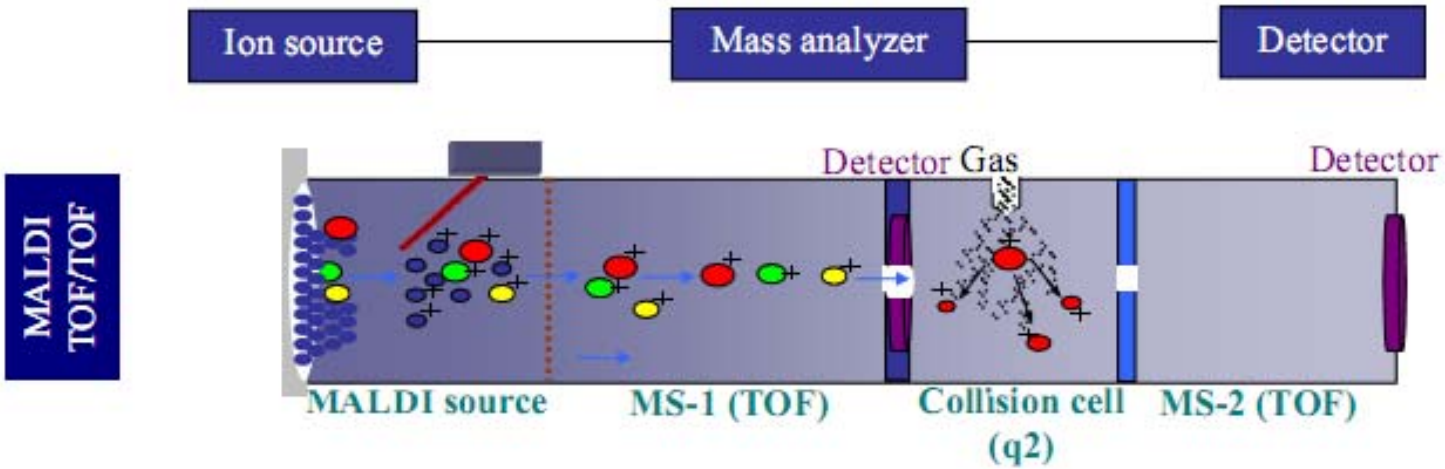
Protein Identification Using Mass Spectrometry



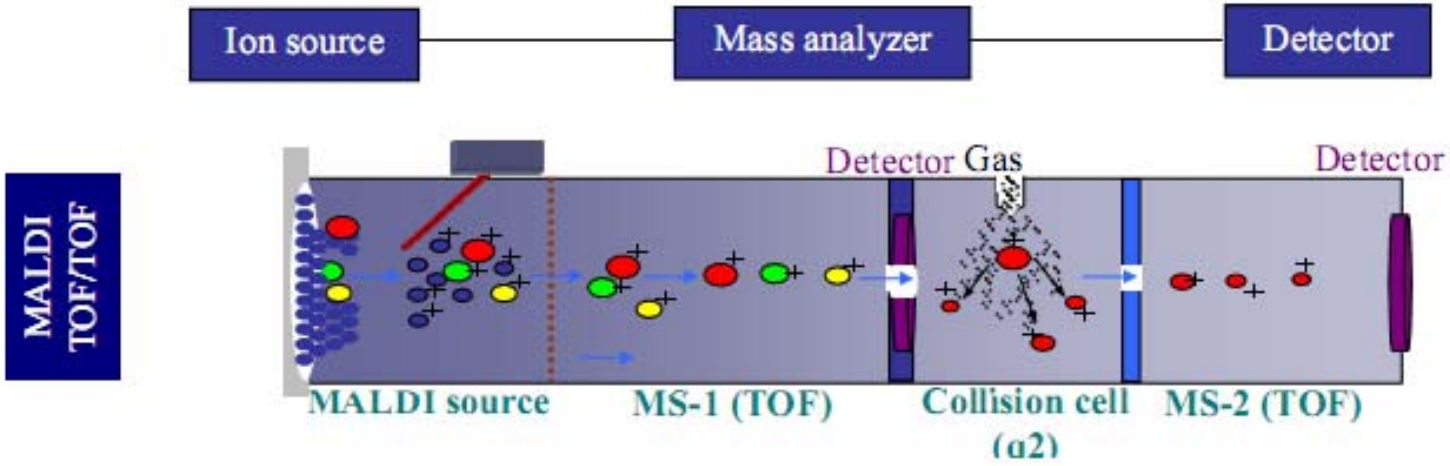
Protein Identification Using Mass Spectrometry



Protein Identification Using Mass Spectrometry



Protein Identification Using Mass Spectrometry



P2 / m → F1 / m1 (TK), F2 / m2 (STK), F3 / m3 (NSTK), F4 / m4 (HNSTK), F5 / m5 (EHNSTK) → P2 / EHNSTK

Proteomics in ABRII

Sample preparation

↓

Protein extraction

↓

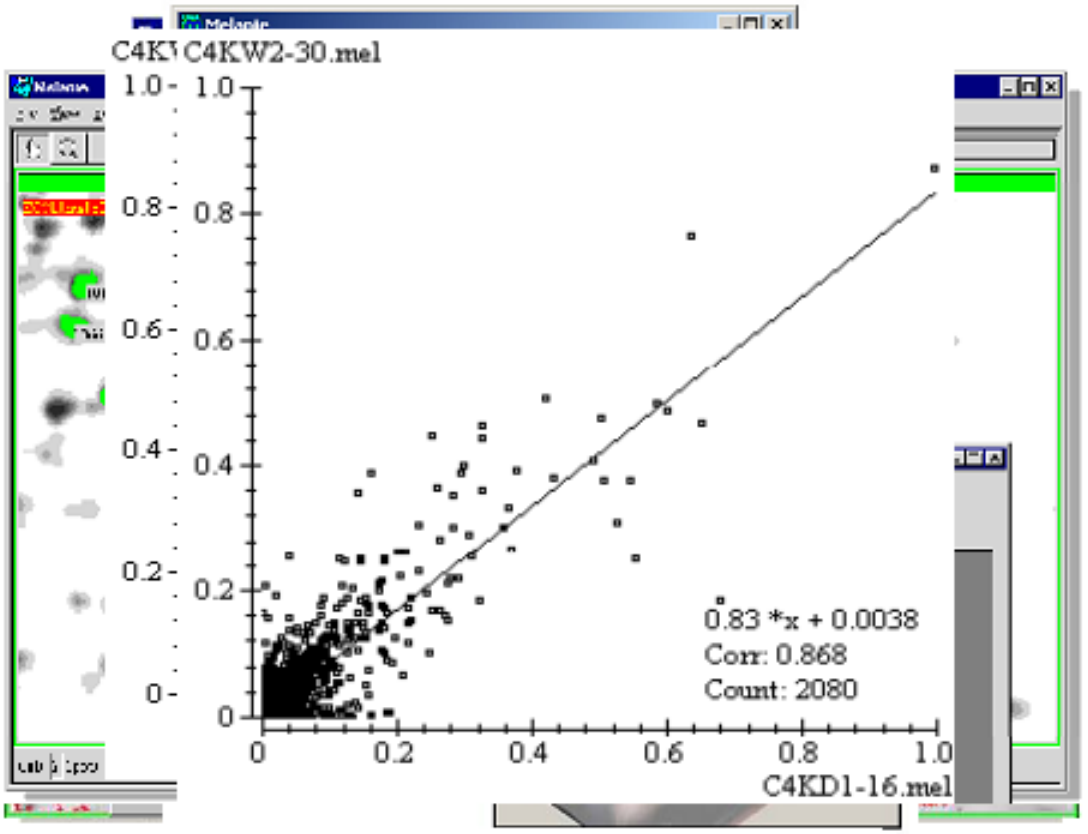
1st dimension (IEF)

↓

2nd dimension (SDS-PAGE)

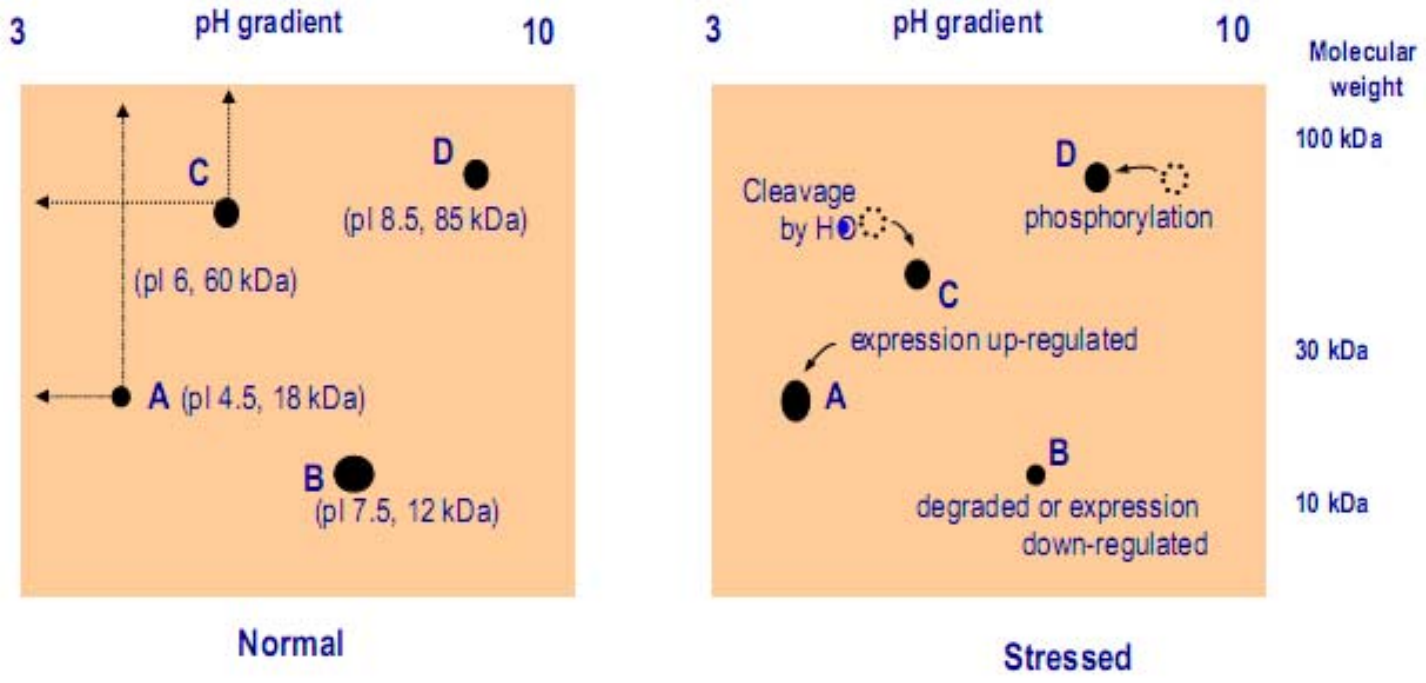
↓

Gel analysis

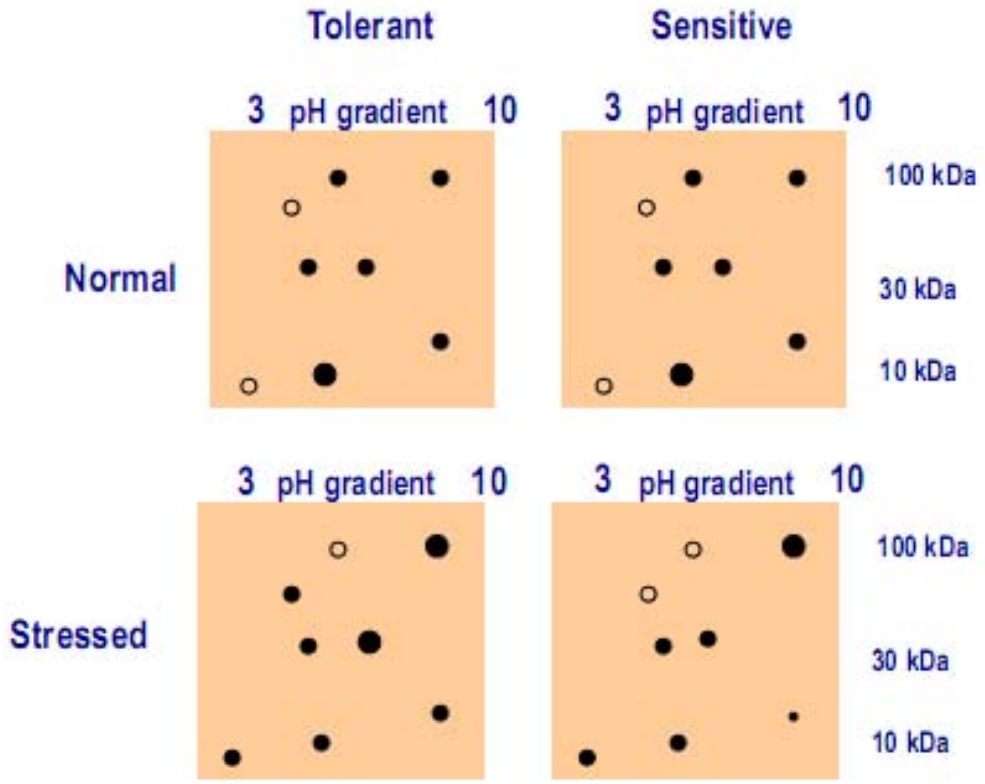


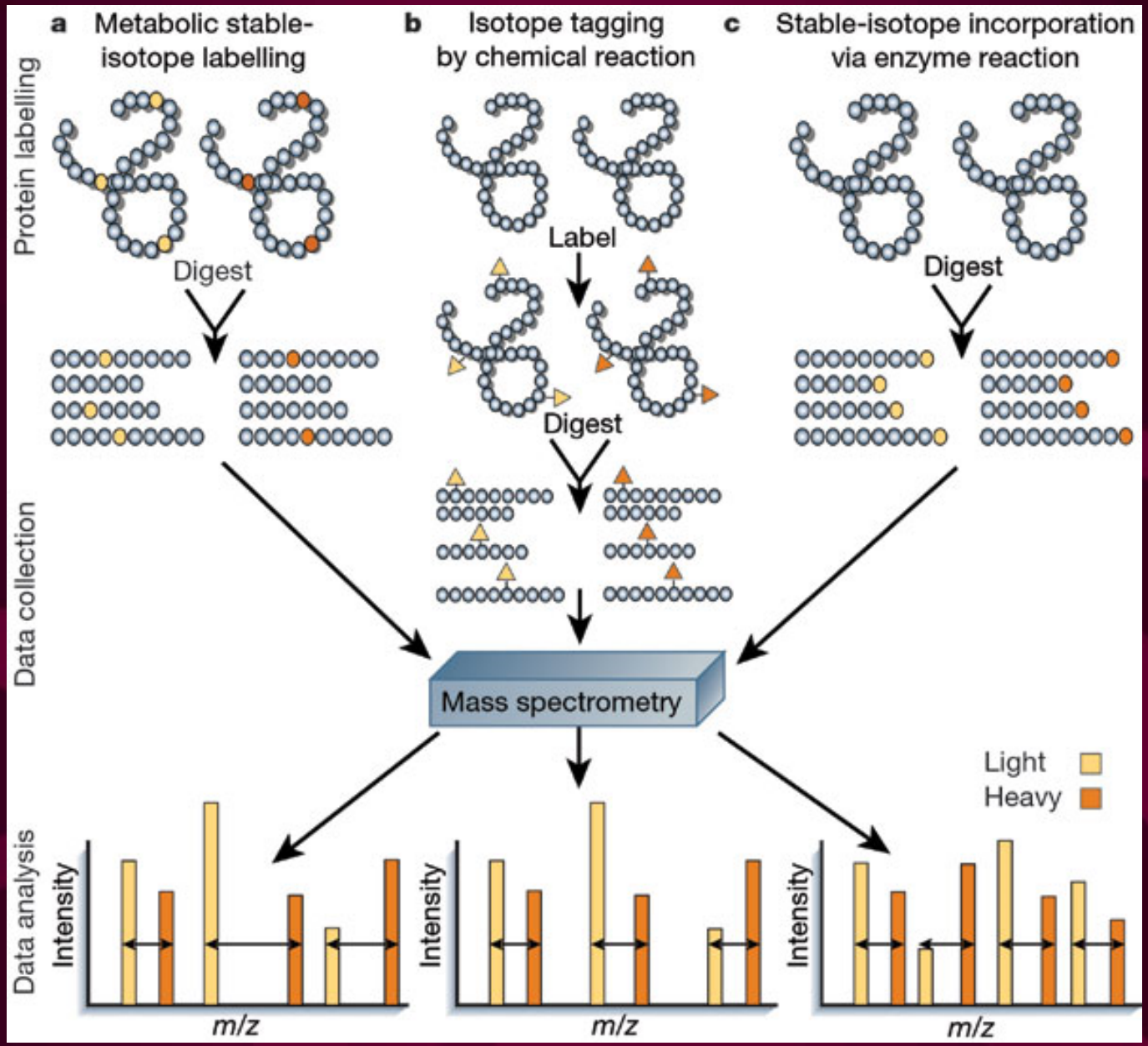
a well-watered plant and a drought-stressed plant

Expression pattern



Expression pattern




a Metabolic stable-isotope labelling
b Isotope tagging by chemical reaction
c Stable-isotope incorporation via enzyme reaction
Protein labelling
Digest
Label
Digest
Digest
Data collection
Mass spectrometry
Light
Heavy
Data analysis
Intensity
m/z
Intensity
m/z
Intensity
m/z

Amino Acid Masses

Amino acid	Mass(avg)	Amino acid	Mass(avg)
G	57.0520	D	115.0886
A	71.0788	Q	128.1308
S	87.0782	K	128.1742
P	97.1167	E	129.1155
V	99.1326	M	131.1986
T	101.1051	H	137.1412
C	103.1448	F	147.1766
I	113.1595	R	156.1876
L	113.1595	Y	163.1760
N	114.1039	W	186.2133

Proteomics in ABRII

Sample preparation

↓

Protein extraction

↓

1st dimension (IEF)

↓

2nd dimension (SDS-PAGE)

↓

Gel analysis

↓

Spot excision

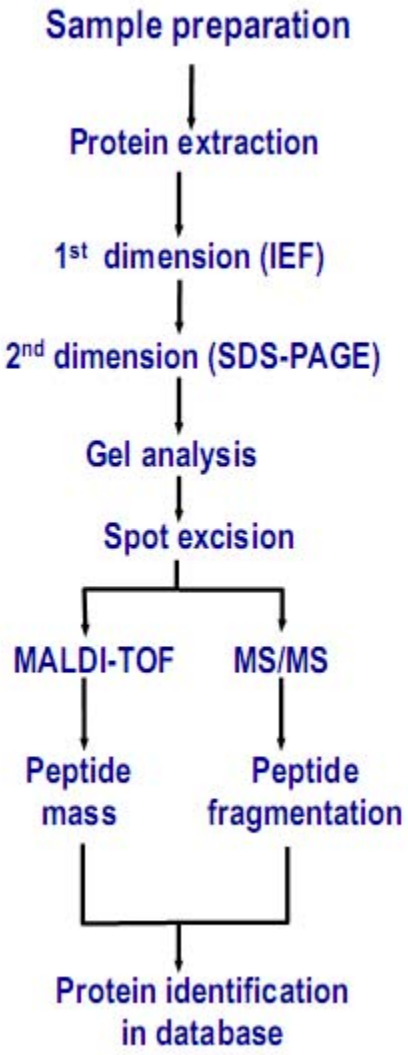
Denature

MITGIQITKAANDLLNDSFRLLDSKGEACIVAAGYAEVVSREYPQLTIVSGQQRFNSLTPSL

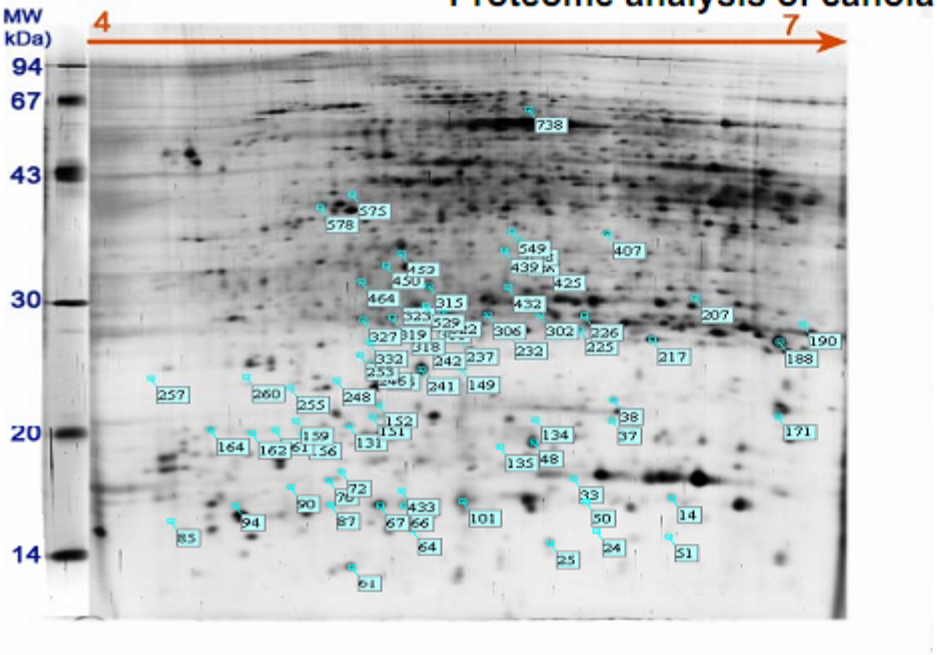
Digest

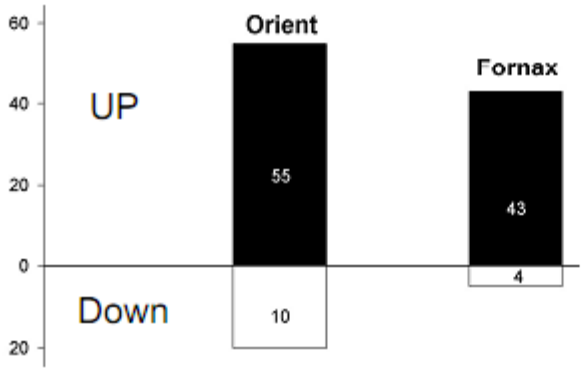
MITGIQITK	AANDLLNDSFR	LLDSK	GEACIVAAGYAEVVSR	EYPQLTIVSGQQR	FNSLTPSL
p1	p2	p3	p4	p5	p6

Proteomics in ABRII



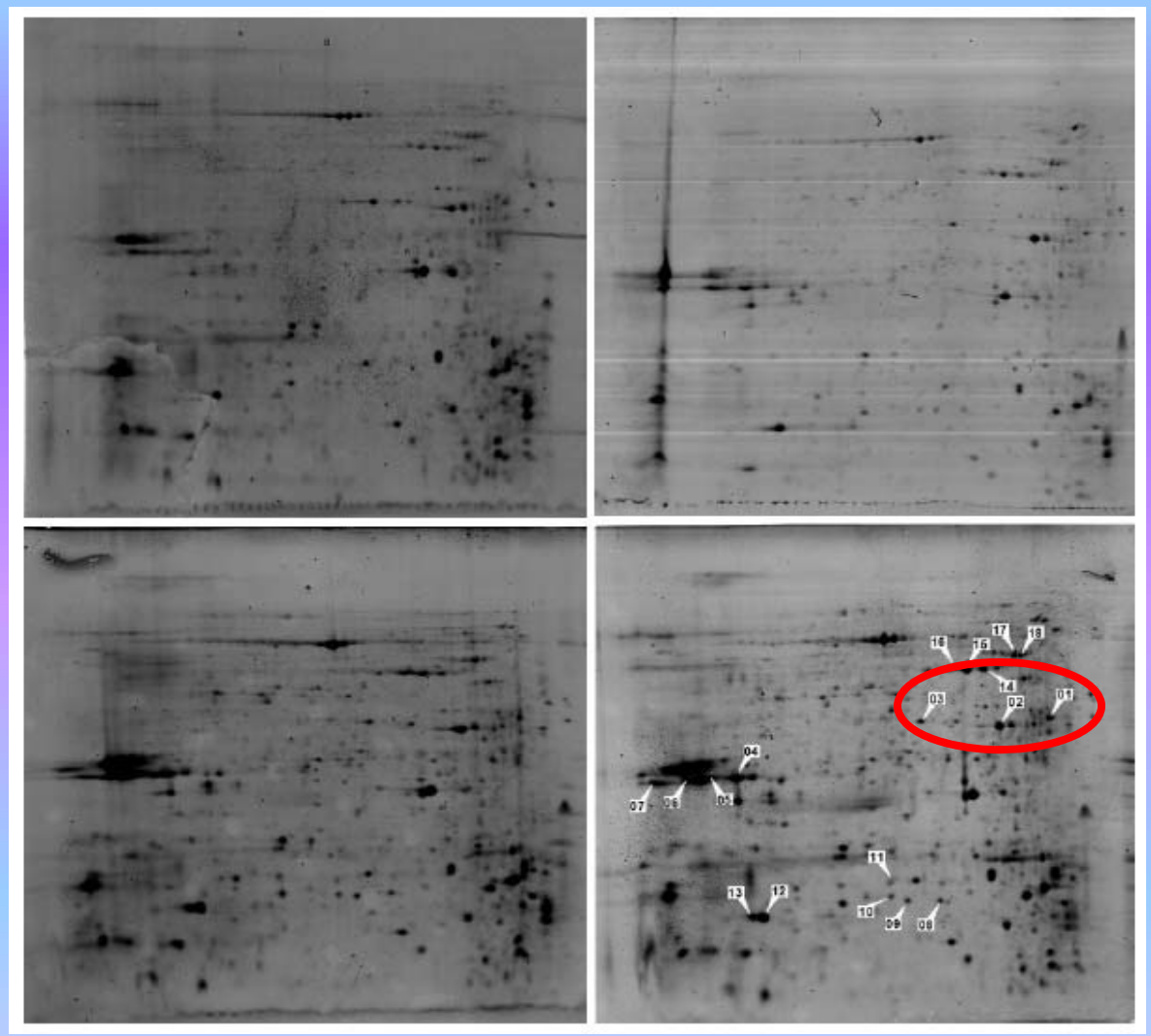
Proteome analysis of canola root





تیمار کنترل

تیمار شوری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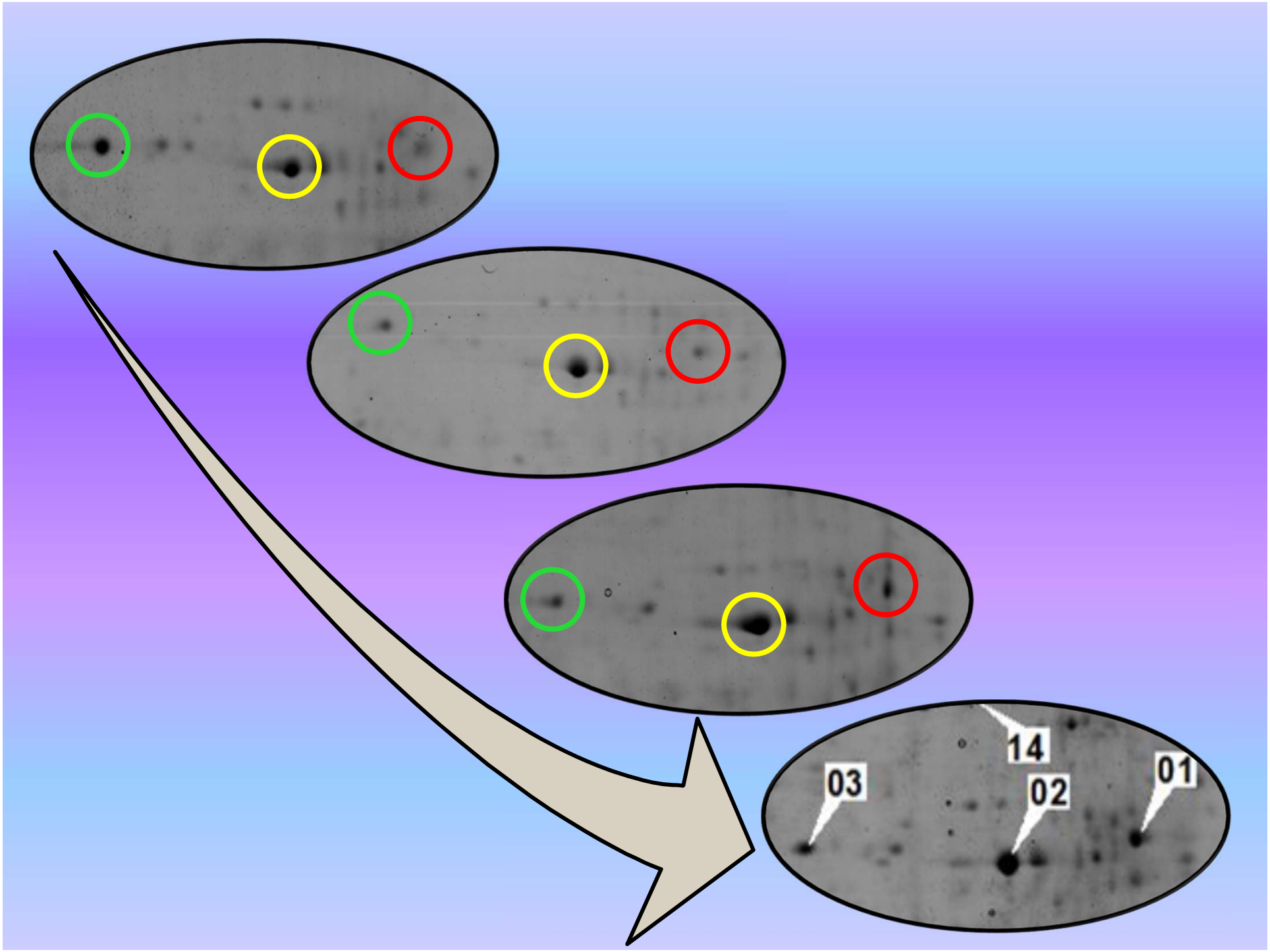
14
03
02
01